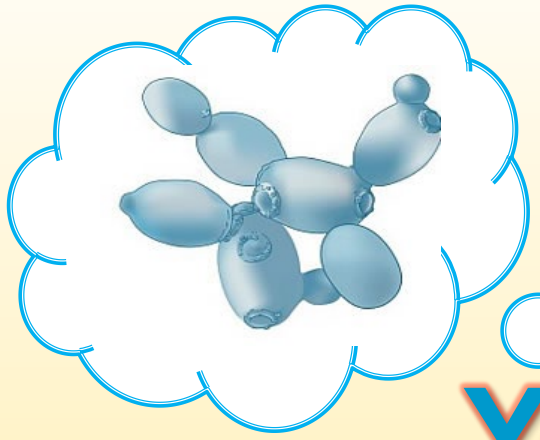
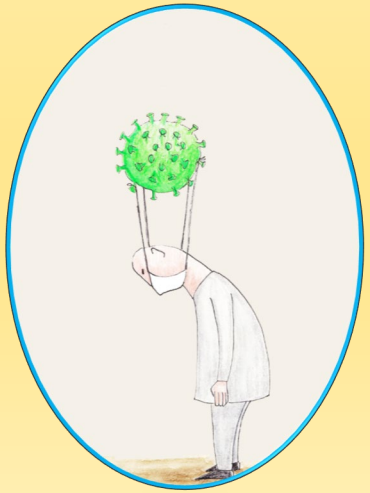


Yeast System as Producer

Antimicrobial Metabolites

Dr. habil. Anna Salek, August 2024



Protein therapeutics

Protein therapeutics is revolutionizing medicine in the treatment of a wide array of diseases. By leveraging the power of protein engineering, protein therapies can be designed and refined to target disease mechanisms with remarkable precision. Targeted protein degradation further enhances this potential by selectively eliminating harmful proteins, addressing conditions once thought untreatable. The integration of **artificial intelligence** (AI) into protein recognizing and design is further advancing the field. It is already accelerating the development of natural antibody therapies, enabling the creationof highly specific and effective treatments.

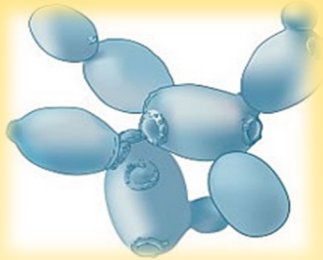
Protein therapeutics

The impact of protein therapeutics is profound, with the promise of addressing diseases ranging from cancers to autoimmune disorders.

As we stand at the dawn of a new era in medicine, our presentation will focus on the latest use of yeast-derived glycoproteins in therapeutics.

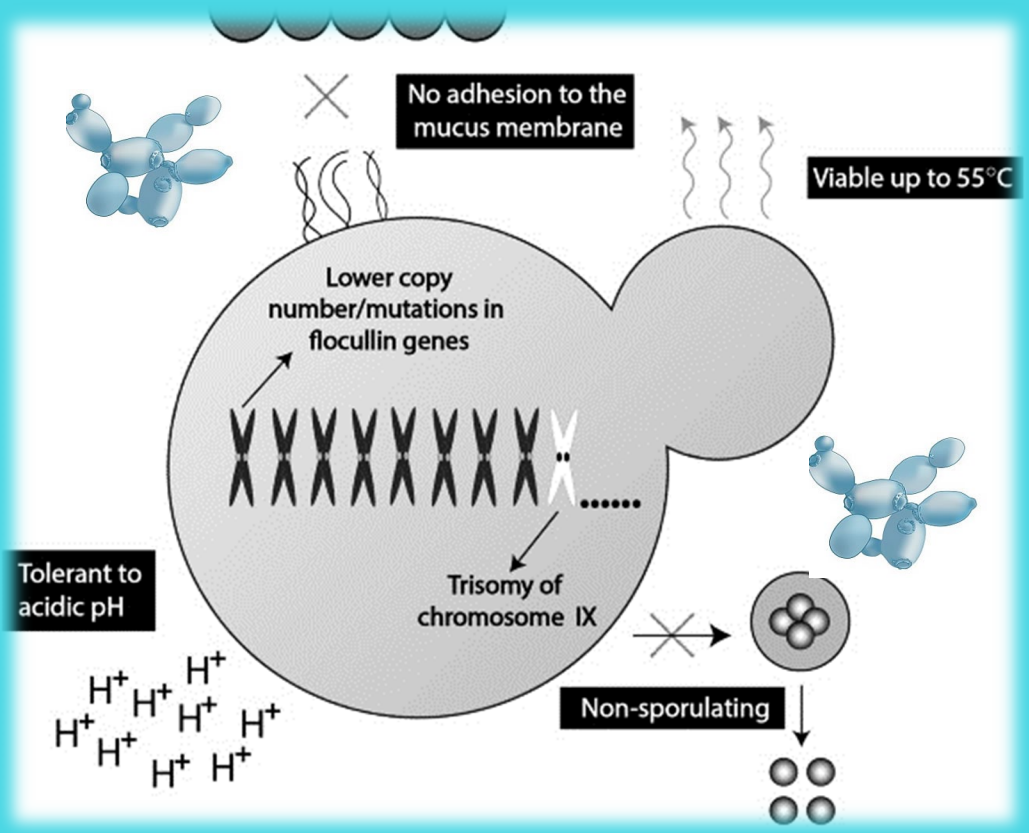
Join us to learn how one aspect of protein therapy is paving the way for personalized treatment through supplementation with naturally derived glycoproteins,

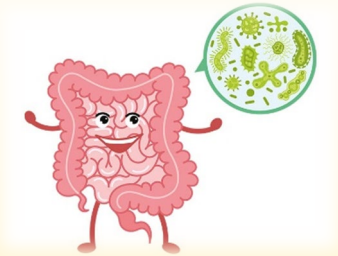
as a new probiotic system from microbioms, like yeasts.



Microbiome

New Therapies



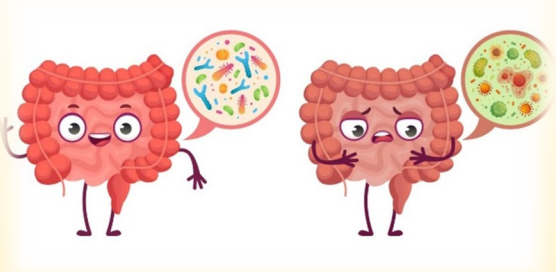


Mikrobiom

Als Mikrobiom bezeichnet man die Gesamtheit aller Mikroorganismen (Bakterien, Pilze und Protozoen), die einen Mikroorganismus (Mensch, Tier, Pflanze) besiedeln. Mikrobiome können u.a. das Immunsystem, den Stoffwechsel und das Hormonsssystem ihres Wirts beeinflussen.

Durch ein besseres Verständnis der Rollen und Funktionalitäten dieser Mikroorganismen ist es möglich, neue Klassen von therapeutischen Präparaten wie lebende Biotherapeutika, Probiotika und Biosimilars zu entwickeln und zu produzieren, welche darauf abzielen, das Gleichgewicht des Ökosystems Mikrobiom wiederherzustellen.

Produkte, die auf lebende Mikroorganismen basieren, wirken zudem in der Regel nicht direkt, d. h. auf bestimmte Organe oder Gewebe, sondern interagiere i einem multifaktoriellen Wirkmechanismus.



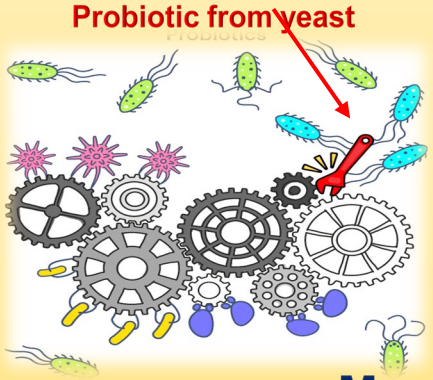
Microbiome

The microbiome refers to the entirety of all microorganisms

(bacteria, fungi and protozoa) that colonize a organism (human, animal, plant).

Microbiomes can influence the immune system, metabolism and hormonal system of their host.

A better understanding of the roles and functionalities of these microorganisms makes it possible



to develop new classes of therapeutic preparations, such as live

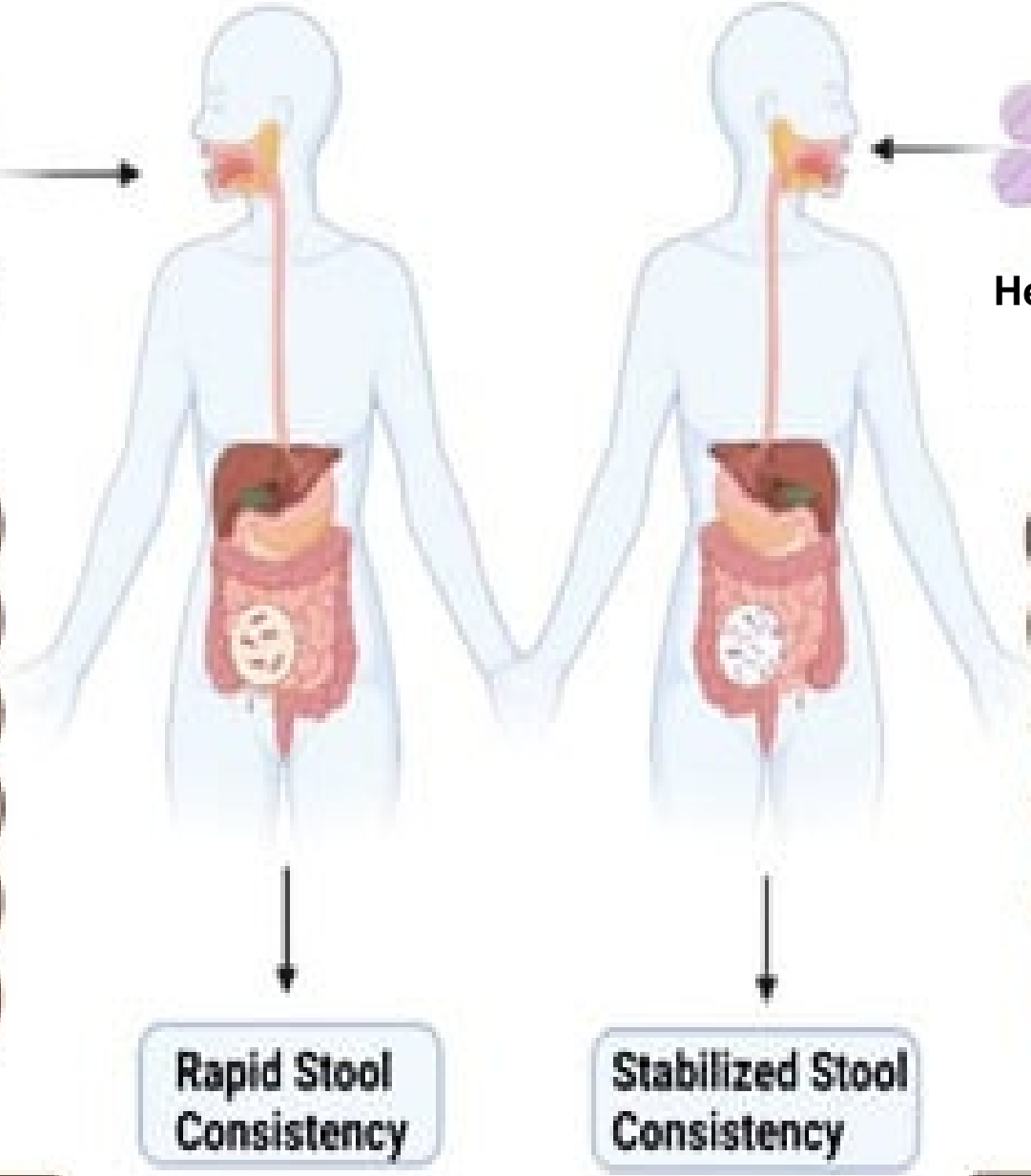
biotherapeutics, probiotics & biosimilars,

which aim to restore the balance of the ecosystem microbiome.

Moreover, products based on living microorganisms generally do not act directly,

i.e. on specific organs or tissues, but interact through a multifactorial mechanism of action.

Antibiotics



Hefe *Williopsic mrakii oder Kluyveromyces lactis*
Probiotics

- Antibiotic Associated Diarrhea
- Clostridium difficile infection
- Enteral Nutrition related diarrhea
- Persistent Diarrhea
- Traveler's Diarrhea
- Acute Diarrhea

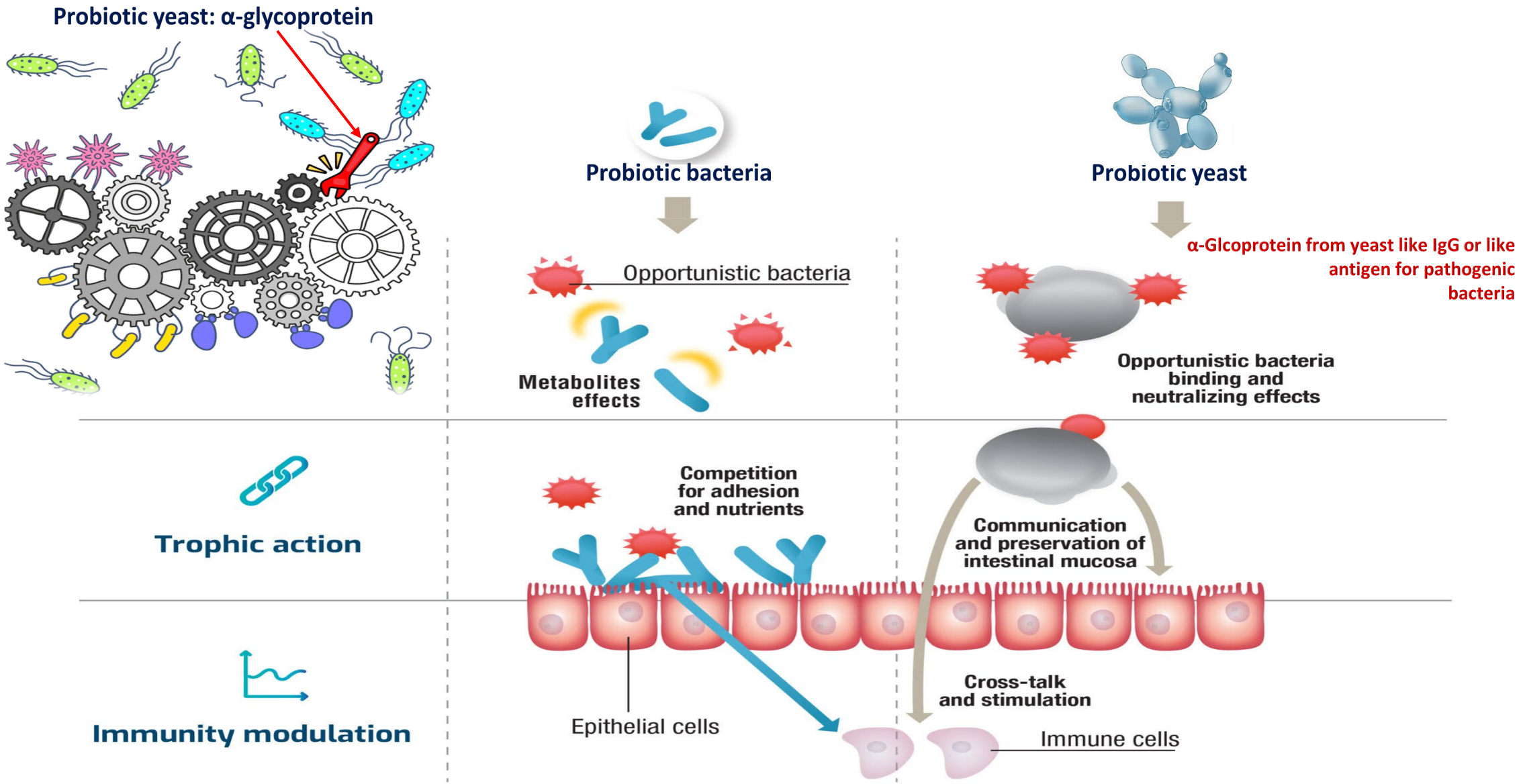
Diseases Caused by Continuous Antibiotics Consumption

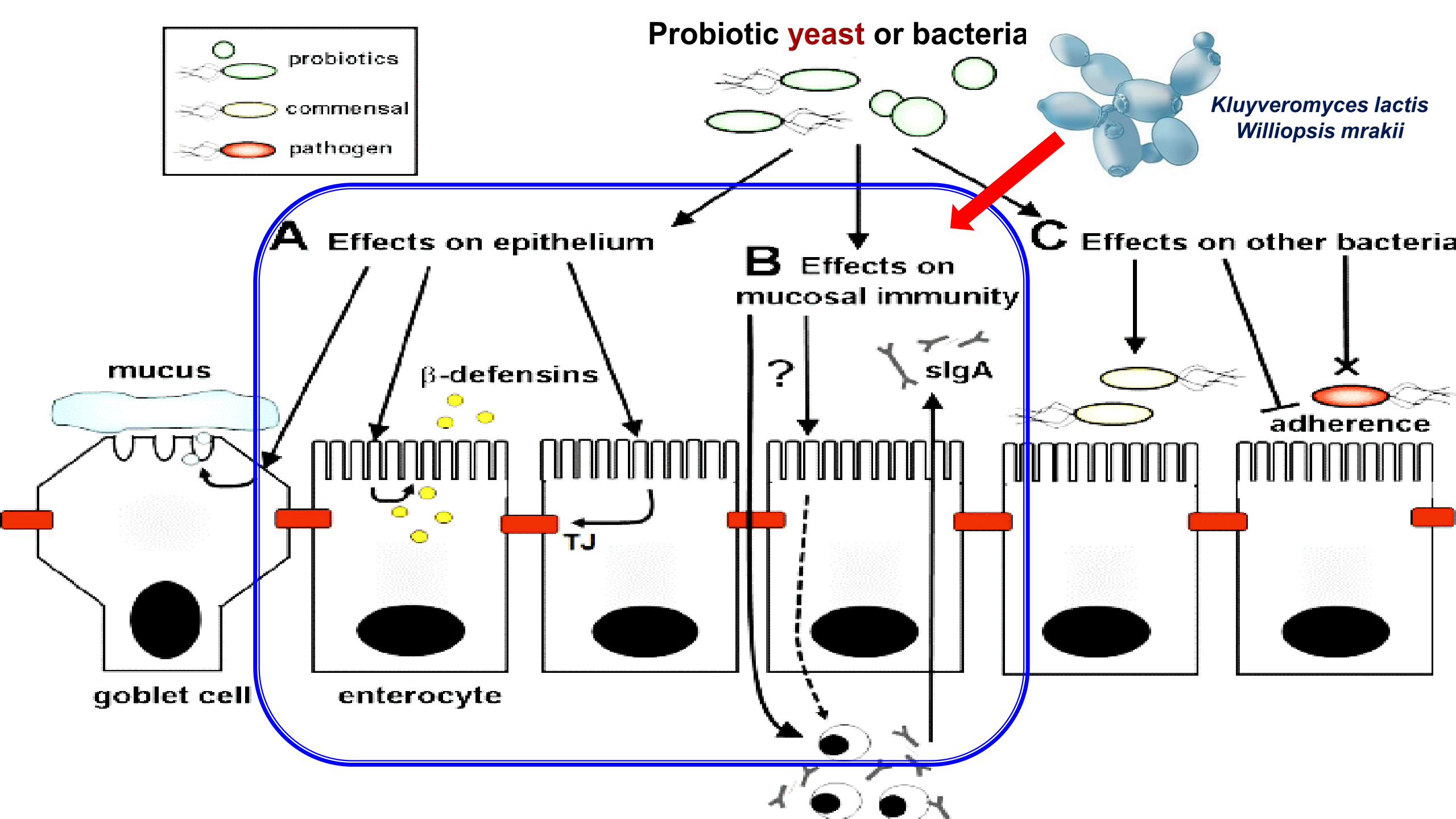
Rapid Stool Consistency

Stabilized Stool Consistency

- Immune System Modulation
- Anti-carcinogenic Effects
- Antiviral Properties
- Antibacterial Properties
- Antioxidant Properties
- Antibiotic Resistance

Beneficial Effects of Probiotic Yeast


Probiotic yeast: α-glycoprotein
Probiotic bacteria
Probiotic yeast
α-Glcoprotein from yeast like IgG or like antigen for pathogenic bacteria
Opportunistic bacteria
Metabolites effects
Opportunistic bacteria binding and neutralizing effects
Trophic action
Competition for adhesion and nutrients
Communication and preservation of intestinal mucosa
Immunity modulation
Epithelial cells
Cross-talk and stimulation
Immune cells

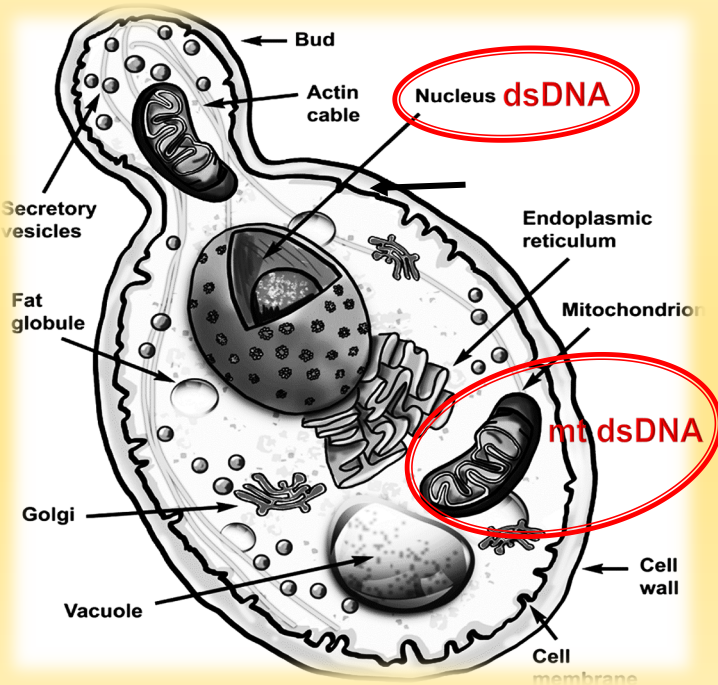

probiotics
commensal
pathogen
Probiotic yeast or bacteria
Kluyveromyces lactis
Williopsis mrakii
A Effects on epithelium
B Effects on mucosal immunity
C Effects on other bacteria
mucus
β-defensins
?
sIgA
adherence
TJ
goblet cell
enterocyte

Yeast Killer System

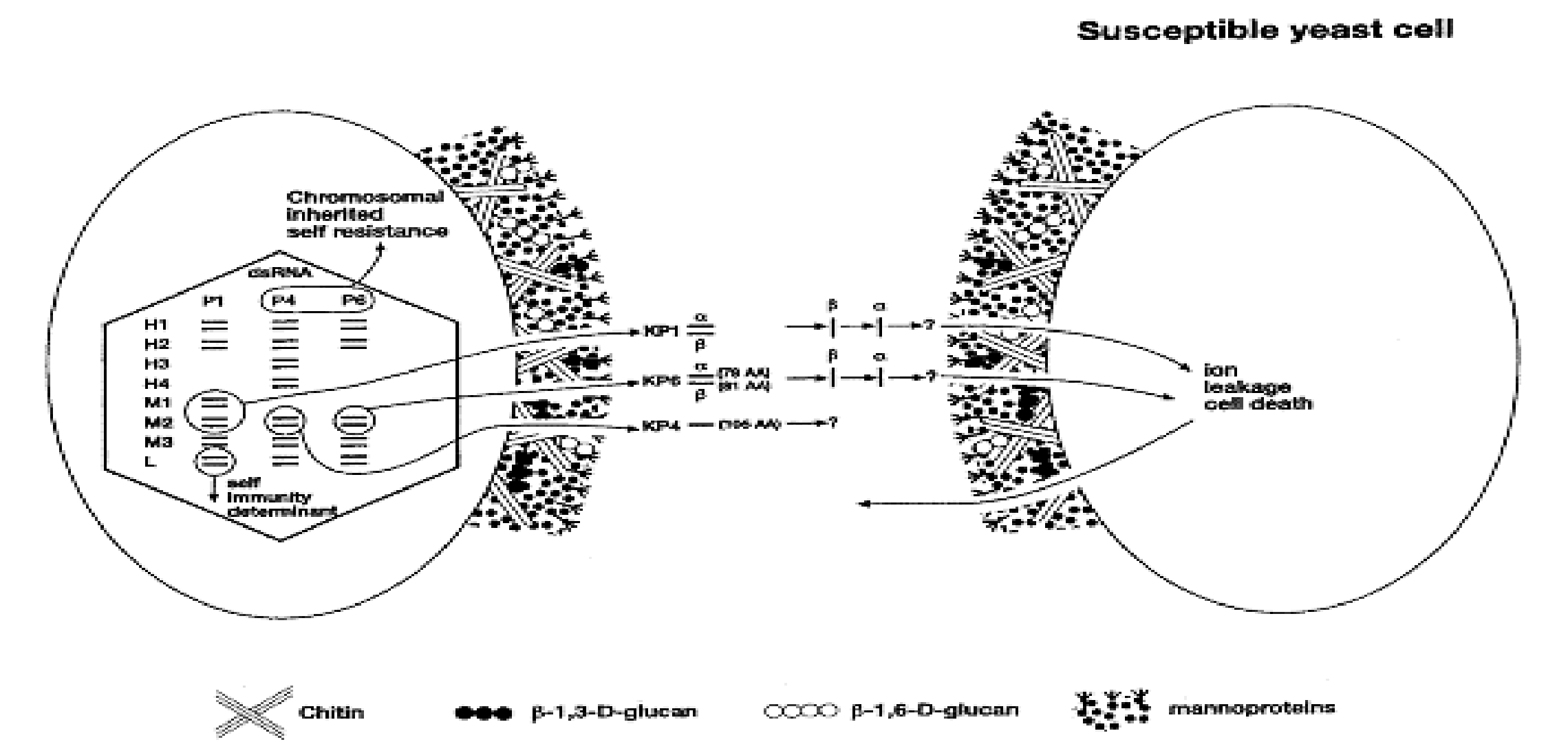
The yeast system has been shown to have advantages over conventional systems as a vaccine vehicle. For example, *Williopsis mrakii* is generally regarded as safe for animals and human beings, secreting Yeast Complex Proteins (YCPs).

Certain methods of genetic engineering, such as electrofusion of the Cell cytoplasm, lead to amplification of the production and secretion of proteins (YCPs, mainly glycoproteins & peptides) with therapeutic properties, i.e. antimicrobial proteins.

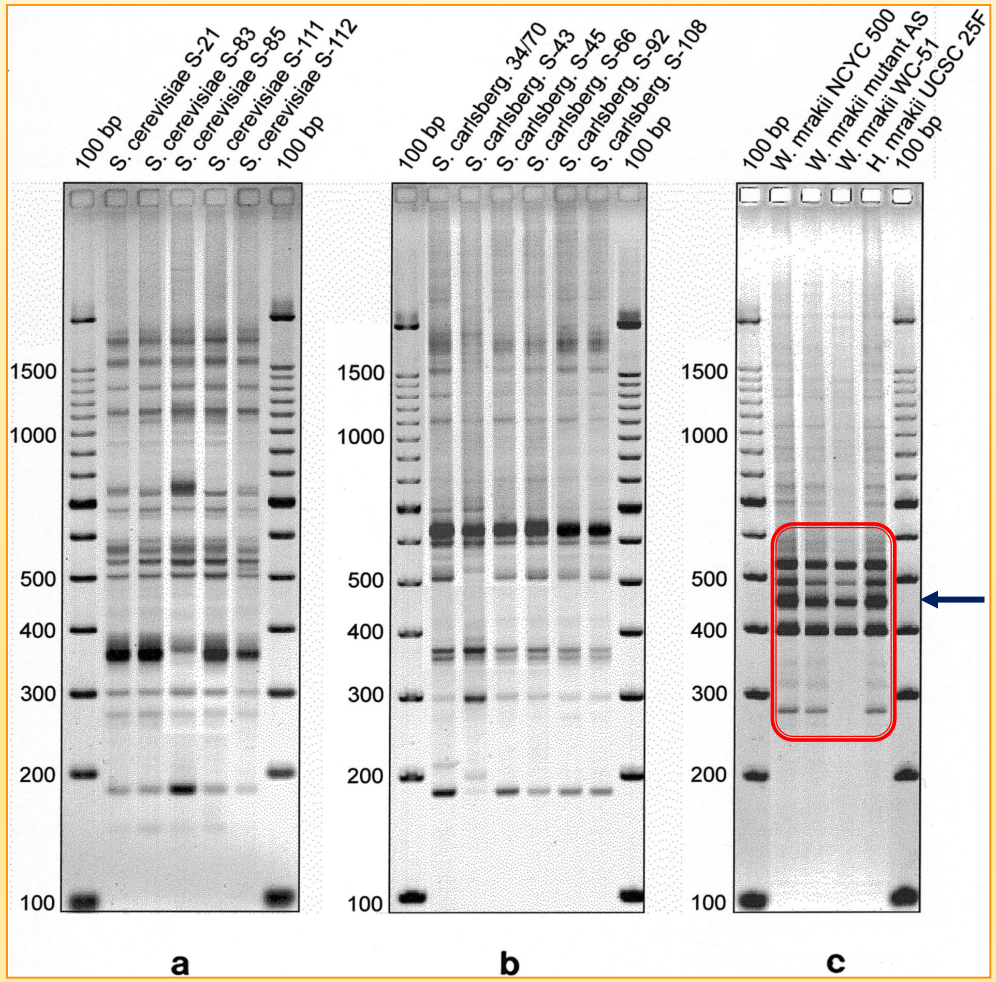
Alfa-glycoproteins (killer proteins) are a type of conjugated proteins with shorter, branched carbohydrate chains known as oligosaccharides.



Killer strain, *Williopsis mrakii* with chromosomal dsDNA

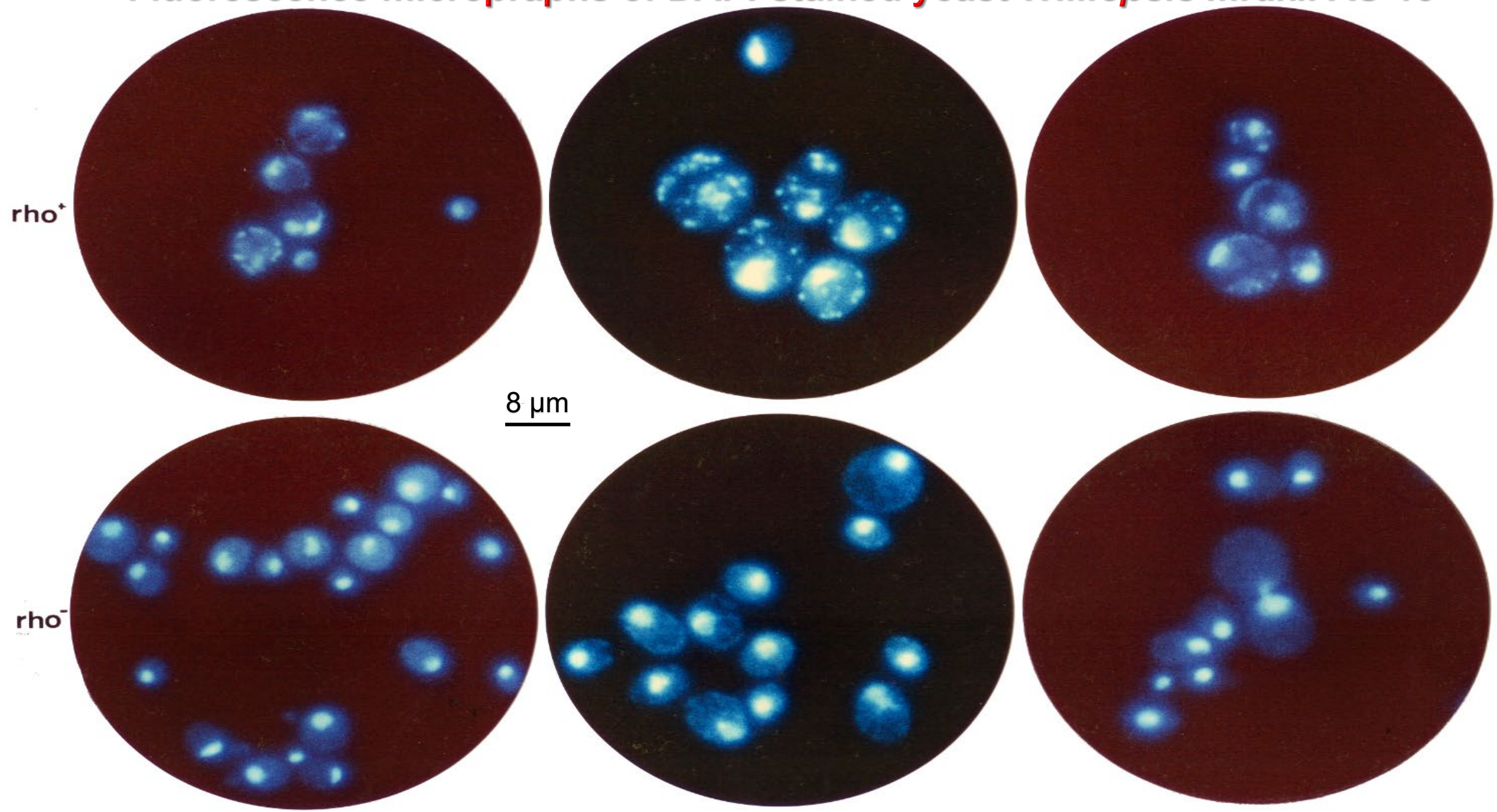


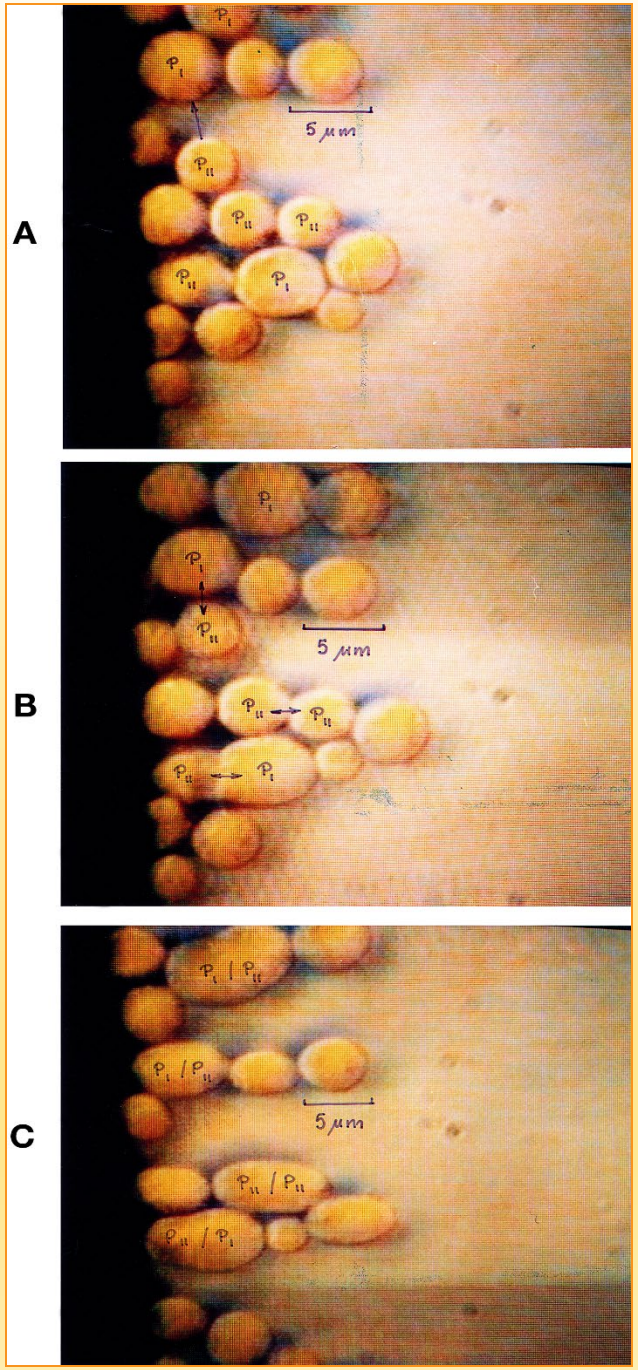
IL-PCR-fingerprints of *S. cerevisiae* and *Williopsis mrakii* generated by IL-primer GR



***Williopsis mrakii* fingerprints**

Fluorescence micropraphs of DAPI-stained yeast *Williopsis mrakii* AS-15





Electrofusion

A. Dielectrophoresis

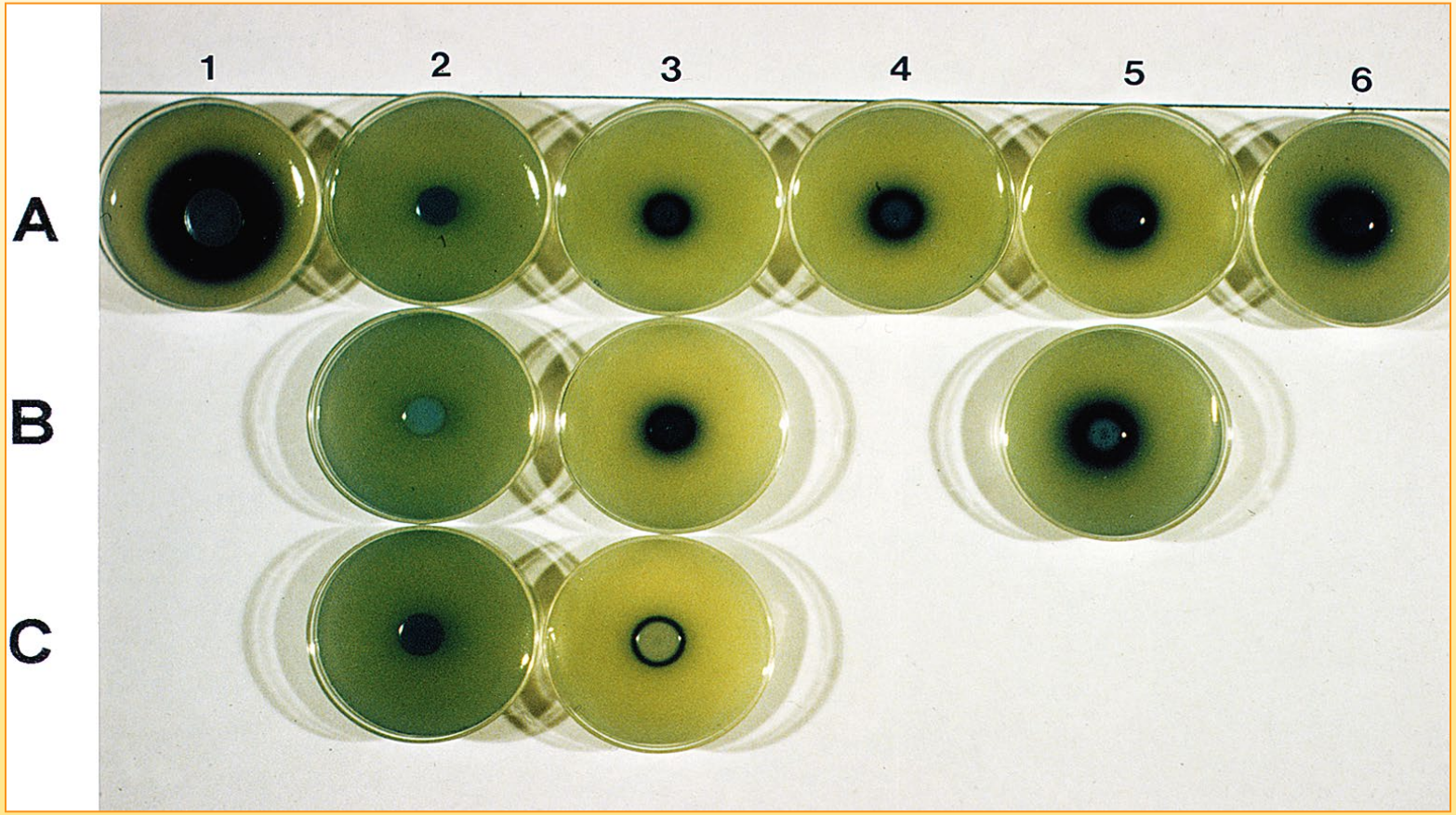
Electrofusion

B. Disturbance of phospholipides

Electrofusion

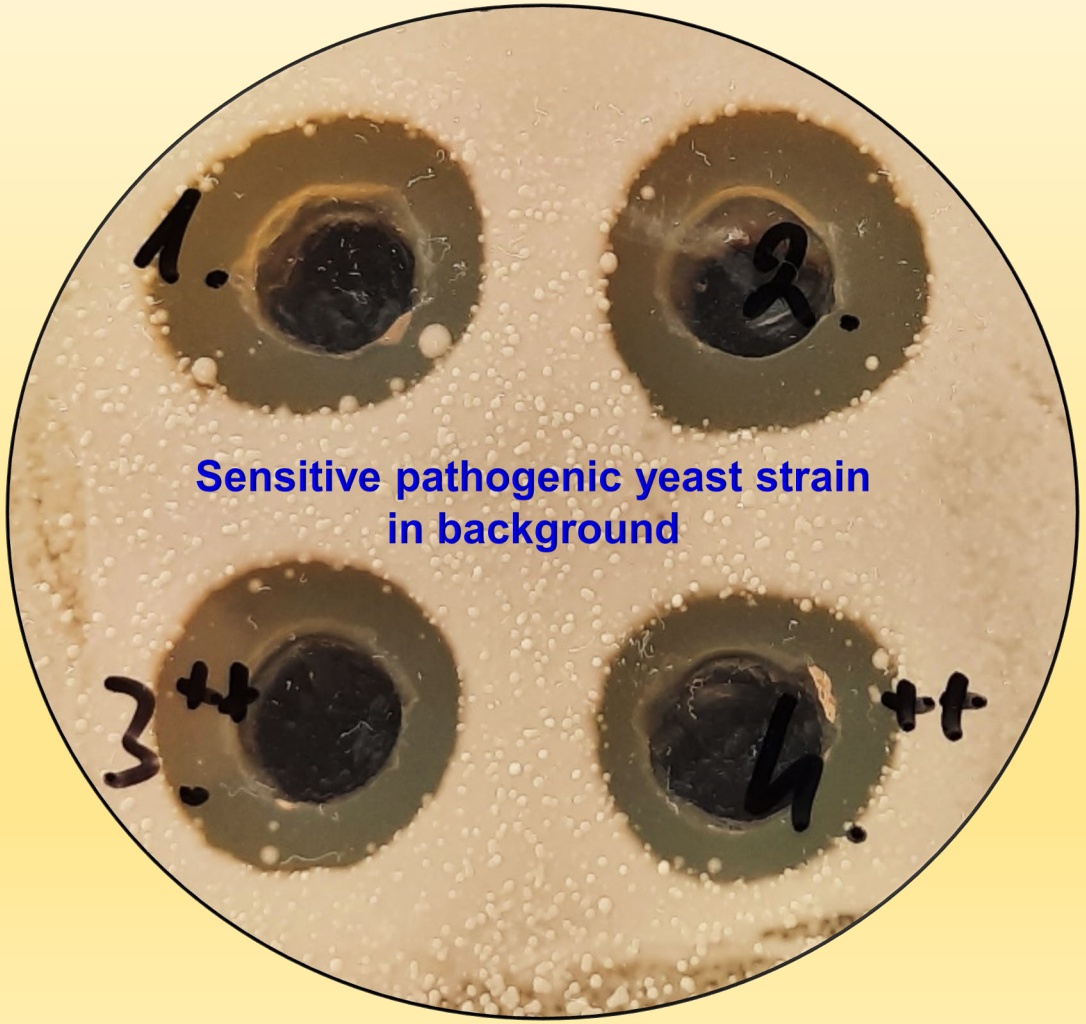
C. Fusion of cytoplasms

Assay of zymocin activity, i.e. killer proteins from *Williopsis mrakii* AS-15



Petri dishes carrying assays for killer activity of single colonies of different yeast strains, incl. *Williopsis mrakii* AS-15

Yeast Complex Proteins from *Williopsis mrakii* AS-15 strains (1, 2, 3, 4) against pathogenic fungi

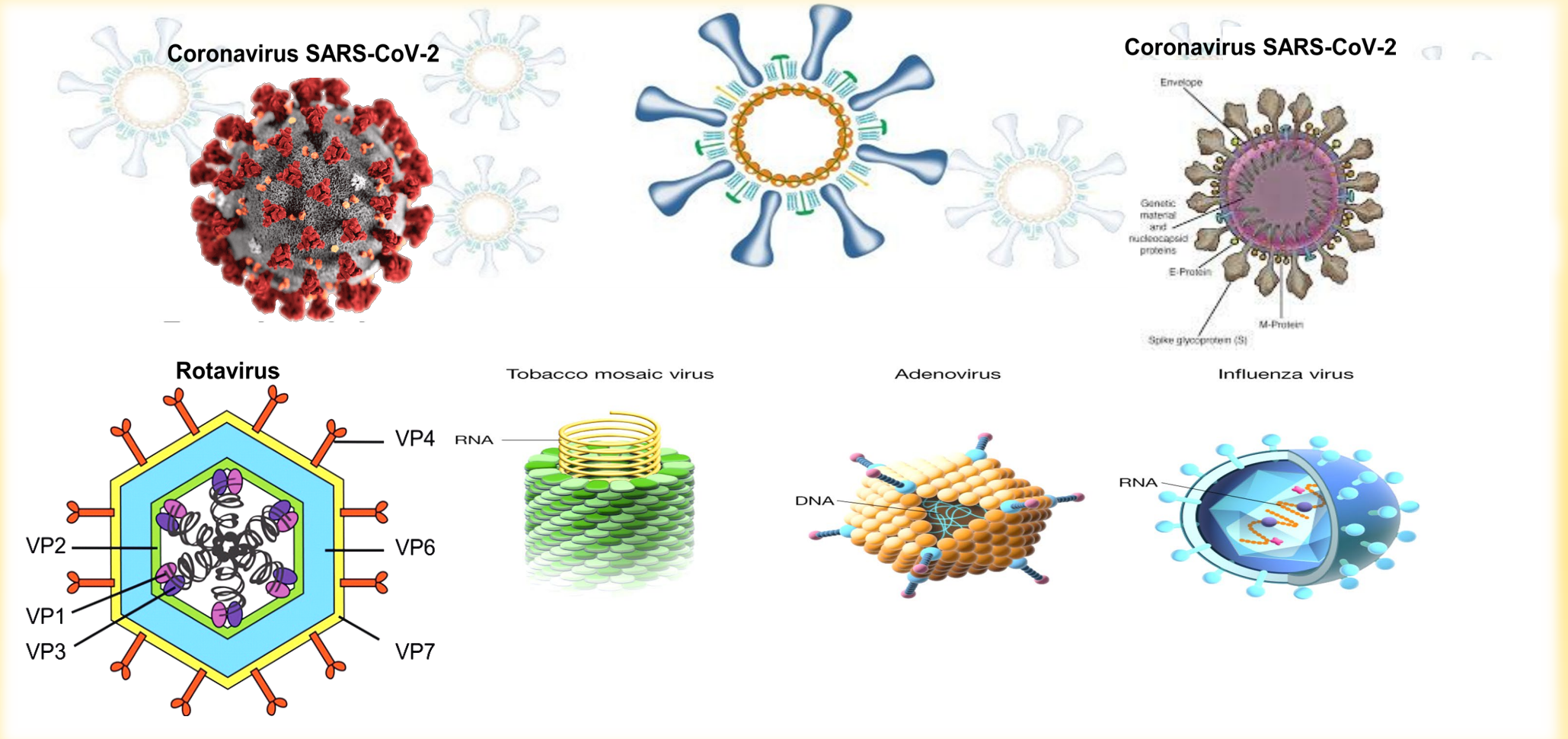


Therapeutic oral medicine (biosimilars)

The killer phenomenon has been reported for strains of the genera *Saccharomyces, Kluyveromyces, Hansenula* (or *Pichia*), *Hanseniaspora, Williopsis, Candida, Torulopsis, Debaromyces, Cryptococcus* and *Ustilago*.

The above-mentioned yeasts produce toxins which act against sensitive strains of the same or closely related species as well as against unrelated microorganisms, including pathogenic microorganisms.

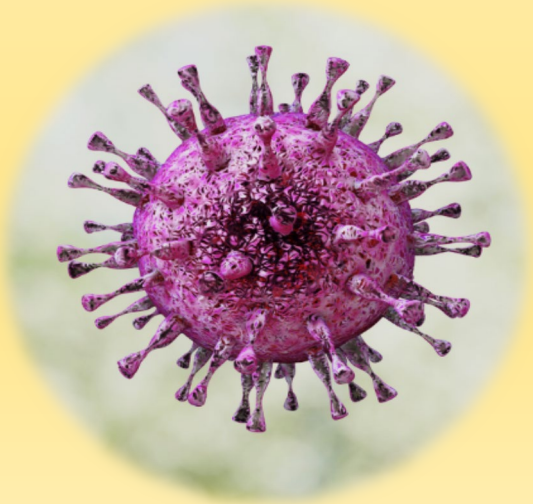
Yeast Complex Proteins *Williopsis mrakii* AS-15 against viruses, e.g. SARS-CoV-2 and others:



Therapeutic oral medicine against SARS-CoV-2

We are presenting an integrative antiviral drug methodology, which combines a systems pharmacology-based network medicine platform that quantifies the interplay between the Coronavirus and host (human macrophage) interaction and beak drug targets in the human network. The basis for that medicine are:

- Yeast killer protein / glycoprotein,
- Specific hydrolases and effector,
- Immunomodulator & antimicrobial metabolit.

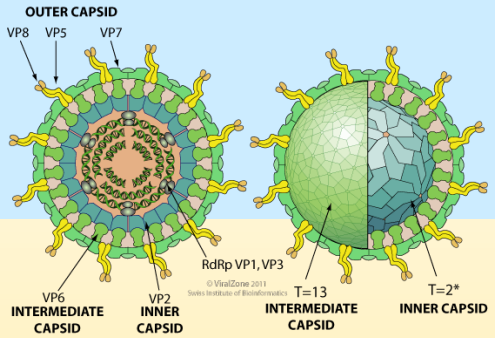


Abstract No. ams0413151934

Field evaluation on the clinical efficacy of Yeast Killer toxins in the treatment of pigs and calves with clinical signs of diarrhoea (enteritis)

Patent Nr. DE 199 12 439.6-09 A1.
„Killer toxins derived from yeast to be used for pharmaceutical manufacturing"

Dr. habil. Anna Salek; Dr. Ferdinand Rott; Prof. S. Donhauser
INROPHARM - vet. pharm. Produkte - GmbH & Co. KG, 94081 Fürstenzell, Germany



Almost 70 % of the diseased patients (piglets and calves)

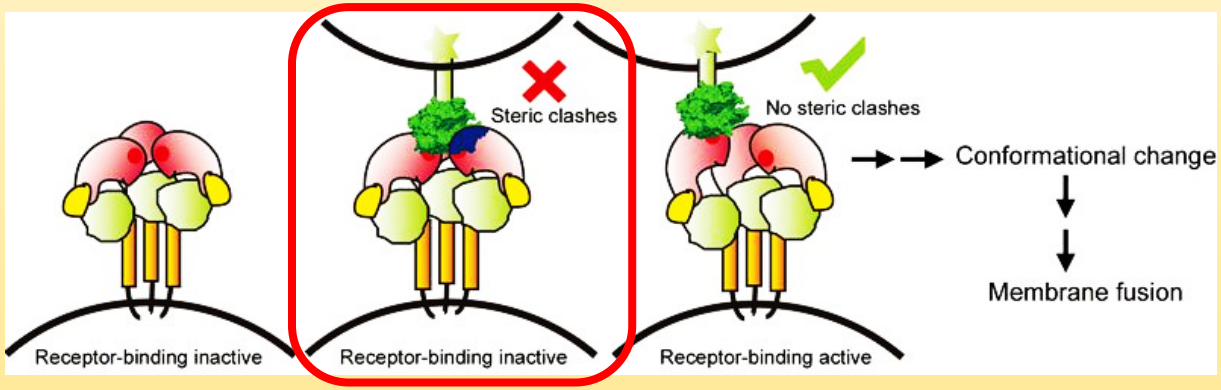
Were housed in farms with endemic intestinal infections. A part of these patients, esp. calves, was pre-treated about 1 – 5 times with antibiotics, but piglets only with killer toxin.

The effect of the treatment with tested killer toxins was mainly positive (80%) in infections suspected to be caused by *E. coli* and viruses (Rota-, Corona virus).

In addition, a reduced effect was observed in infections caused by bacteria *Clostridium.*

Mechanism of neutralisation a spike receptor S1 in SARS-CoV-2 and human receptor ACE2

Some protein, e.g. yeast killer α-glycoprotein from *Williopsis mrakii* AS-15 strain, that functionally associate with Coronavirus (CoV-2) infection, i.e. with spike or envelope, and it can be localized in the subnetwork within the comprehensive human receptor ACE2.

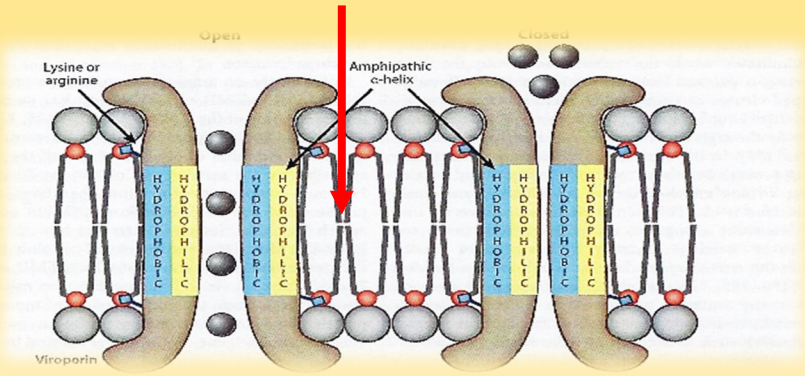


The basis of this mechanism is the specific binding of the corresponding receptors from killer α-glycoprotein together with glycoprotein receptors of S1 spike Coronavirus with high-mannose-content glycan's.

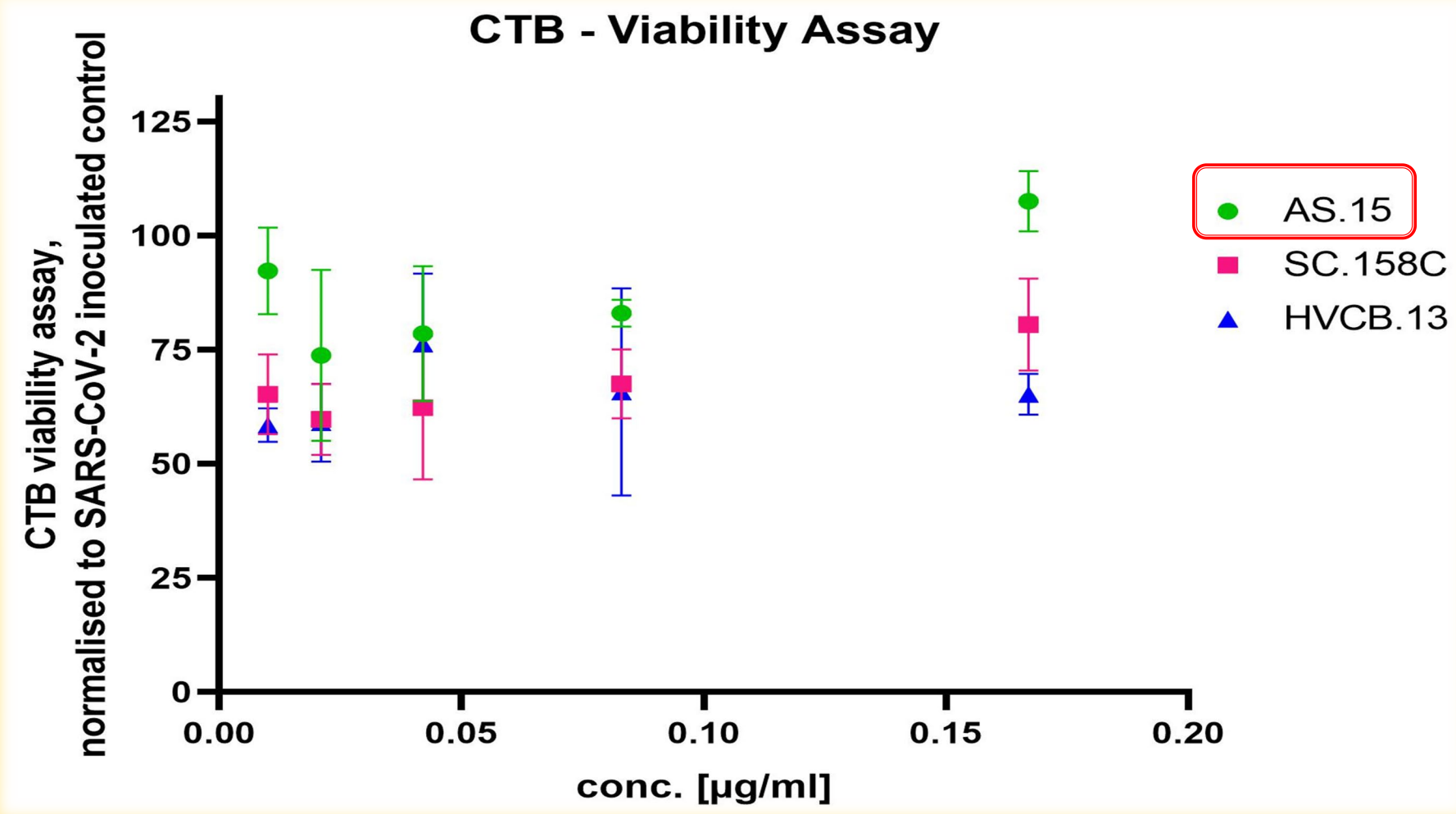
Killer toxin make structural changes in viral (Receptor Binding Domains, RBDs).

Mechanism of neutralisation a protein in envelope of SARS-CoV-2

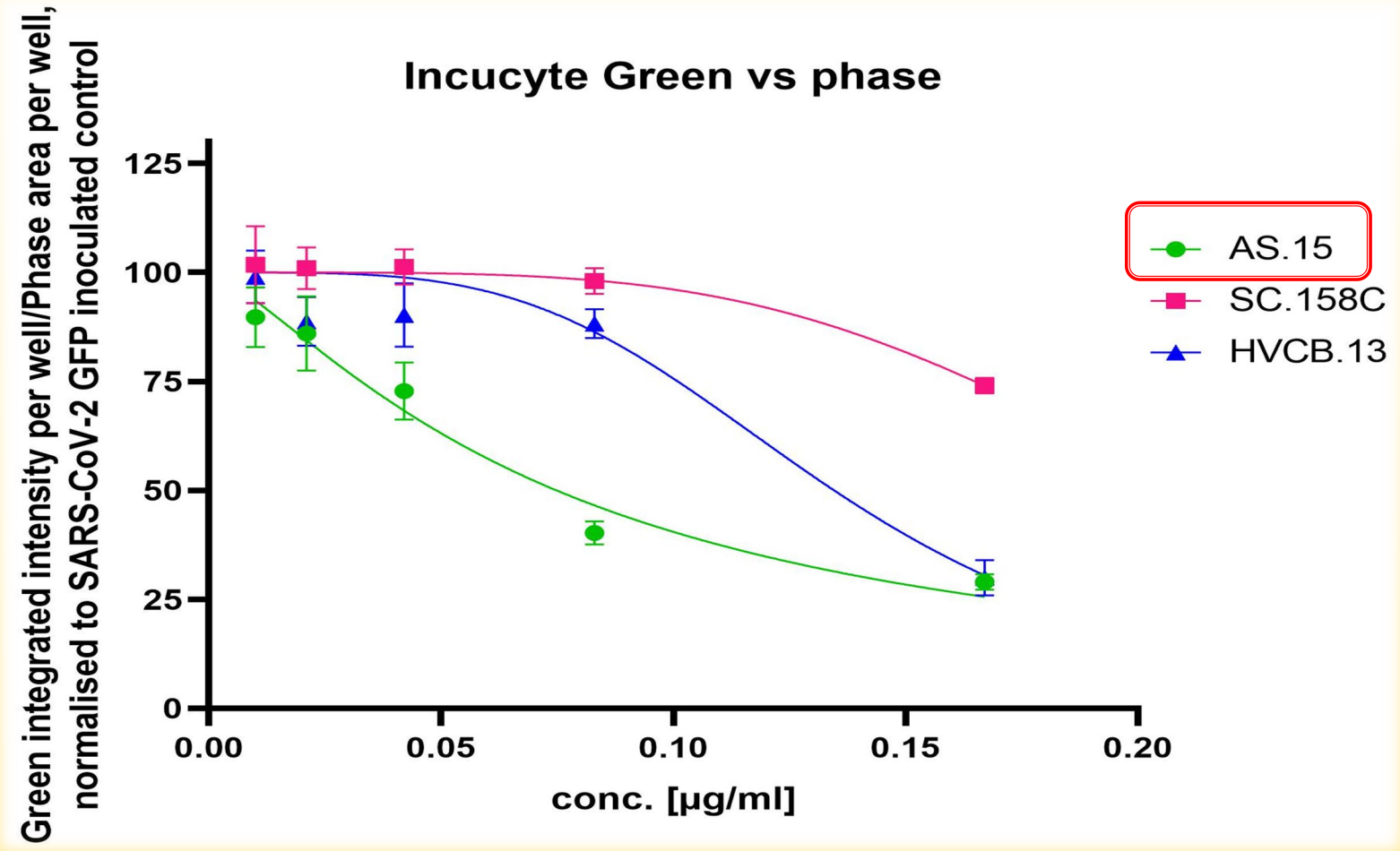
Moreover, the viral ribonucleocapsid is encased within a bilayer lipid envelope containing envelope protein E (one of the main structural proteins of the virus). In addition, single nucleocapsid structure the nucleocapsid is an important subunit for packaging the viral genome (ssRNA) through protein oligomerization. This structure could be destroyed through yeast killer toxins (α-glycoprotein) as well as through competent proteases and biological substances from our oral medicine, which partly destroyed phospholipids bilayer of Coronavirus envelope.



Direct virological test with YCPs from *Williopsis mrakii* AS-15



Direct virological test with YCPs from *Williopsis mrakii* AS-15



The concentrations are calculated using the starting concentration of 1 µg/ml.We see partial inhibition of the infection of up to 70% in the upper concentrations. AS-15 in particular shows a dose-dependent effect even at lower concentrations.

Conclusion

Having regard to the selection of <u>our oral preparation</u>,

which contains specific proteins (α-glycoprotein, like IgG, as antigen)

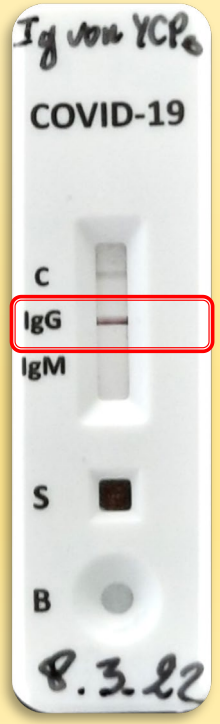
against Coronavirus – would be best medical need,

possibly allowing simultaneous immunization

human organism before SARS-CoV-2 infection.

This preparation could be soon in market.

α-Glicoprotein = IgG

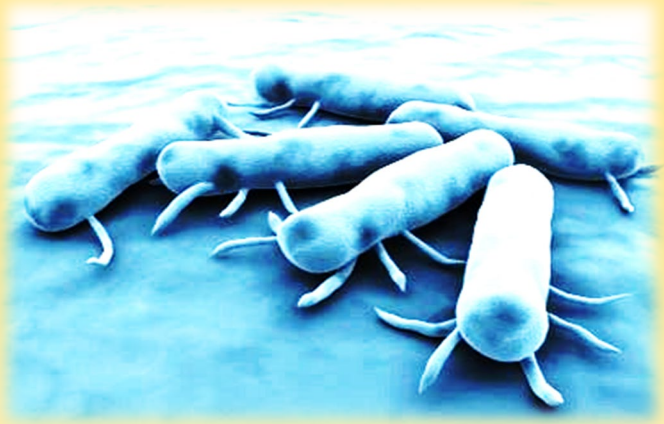


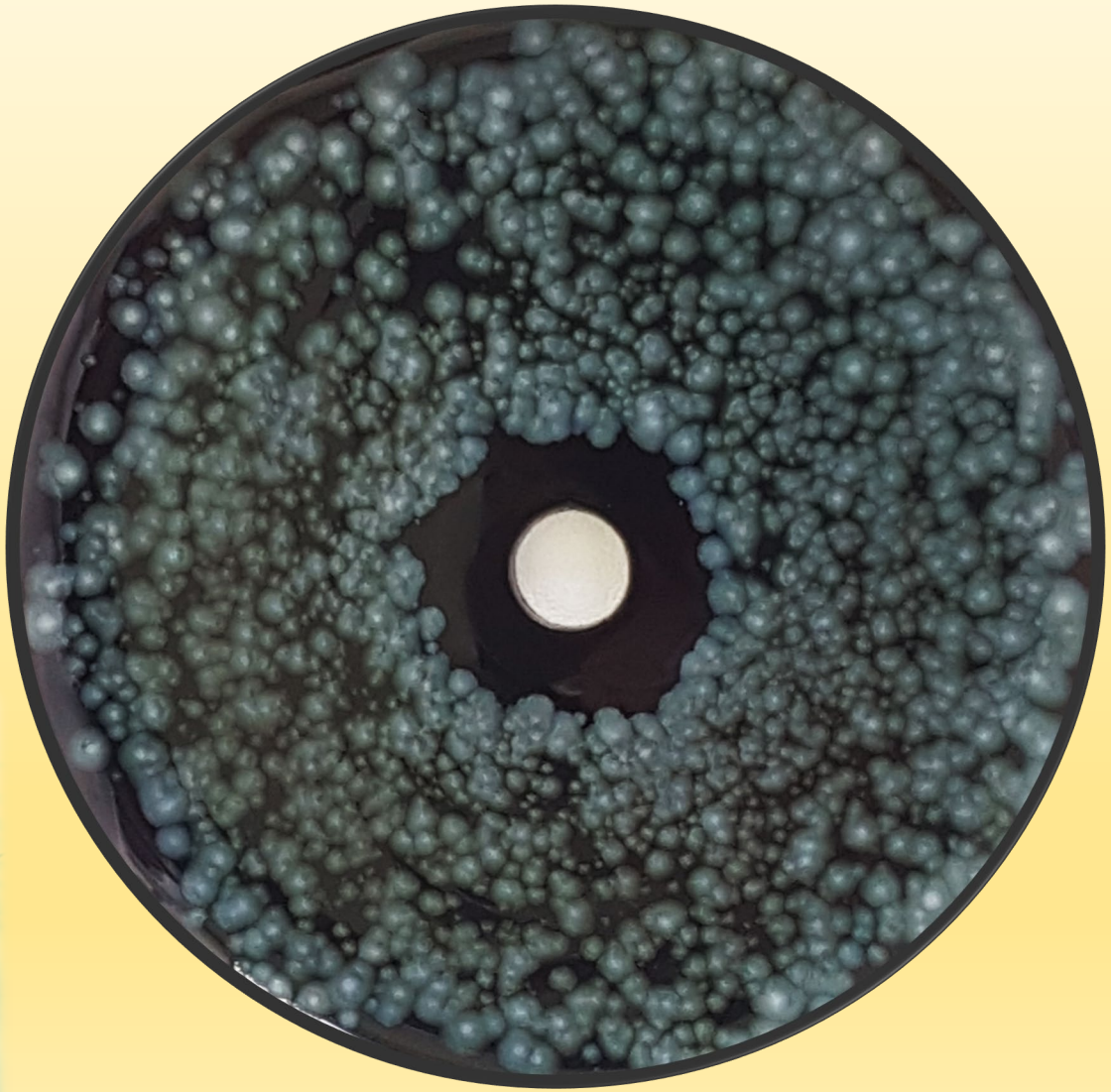
Patent: Salek A.: „Zubereitung sowie pharmazeutische Zusammensetzung zur Anwendung in der Medizin, insbesondere Virologie“ Deutsche Patent, DE 10 2020 001 811 A1, 2021.

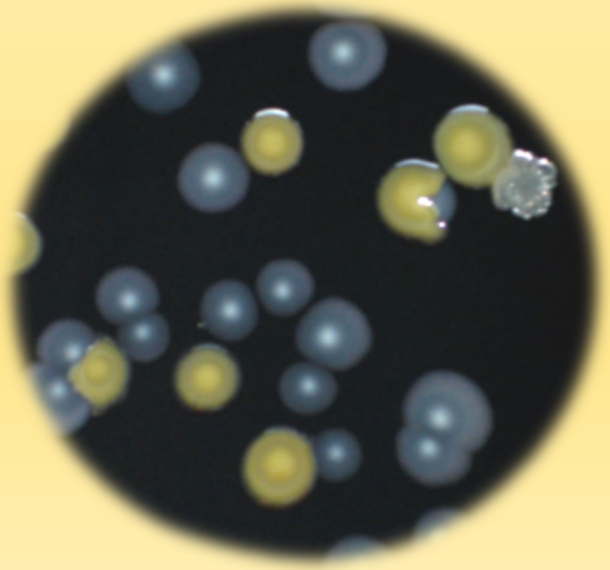
Yeast Complex Proteins(YCPs) from *Williopsis mrakii* AS-15 against bacteria:

- *Legionella pneumophila*
- *Pneumocystis carinii*
- *Enterobacter aerogenes*
- *Pseudomonas aeruginosa*
- *Salmonella spp.*

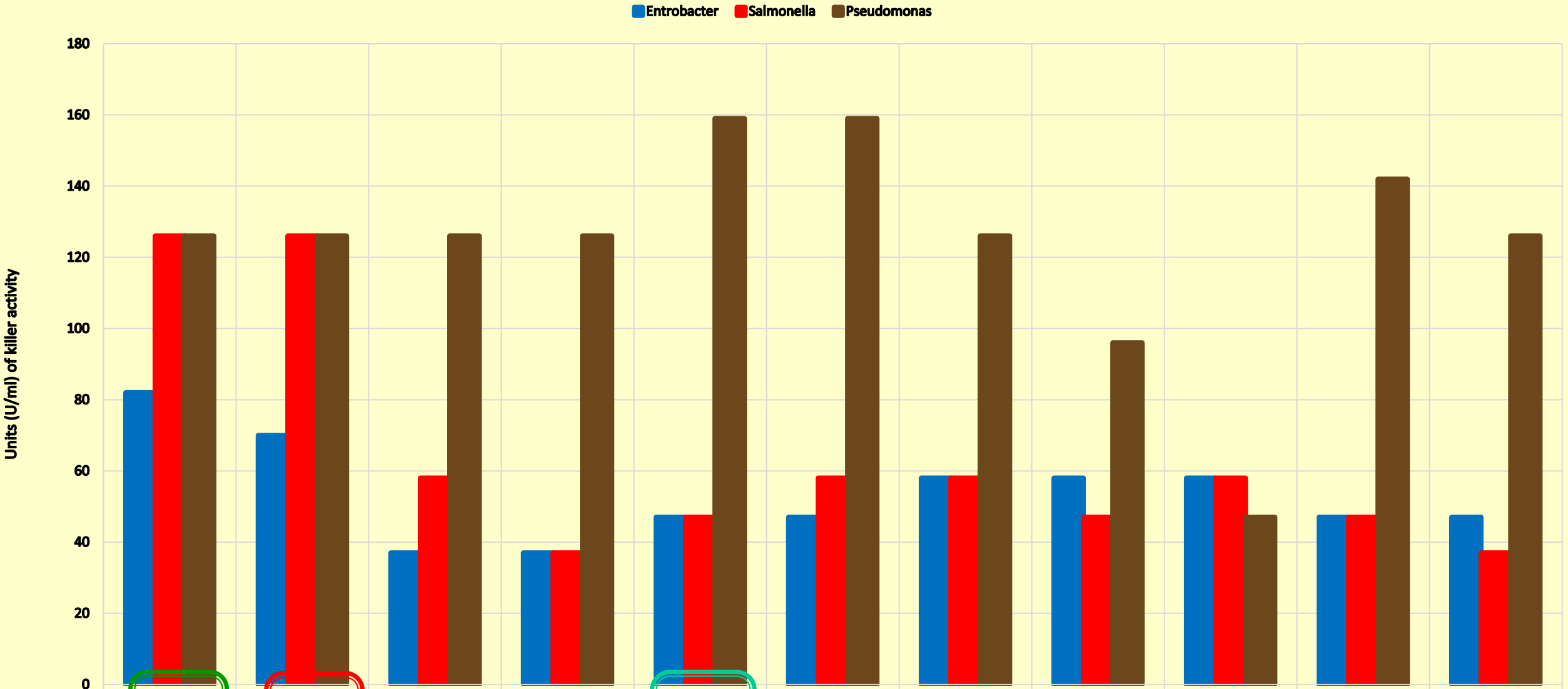
Killer proteins AS-15 destroyed membrane of *Legionella pneumophila*







Influence of YCPs activity on bacteria *Enterobacter* spp., *Salmonella* spp. und *Pseudomonas* spp.



	KT 10x	KT+Guar	KT+Sorbinsäure	KT+Sorb.+Guar	KT+Milchsäure	KT+Milchs.+Guar	KT+Milchs.+Sorb.	KT+Milchs.+Sorb.+Guar	KT+Sorb.+Guar+Raft.	KT+Sorb.+Milchs.+Guar+Raft.	KT+Glycerol
Entrobacter	82	70	37	37	47	47	58	58	58	47	47
Salmonella	126	126	58	37	47	58	58	47	58	47	37
Pseudomonas	126	126	126	126	159	159	126	96	47	142	126

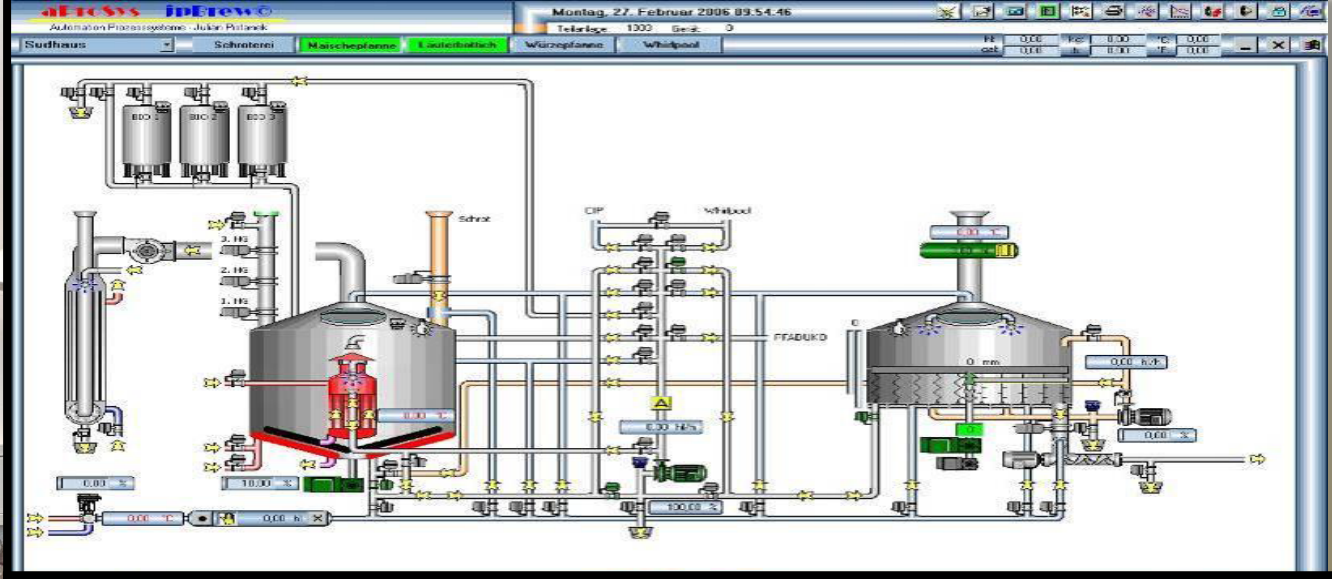
Yeast Complex Proteins

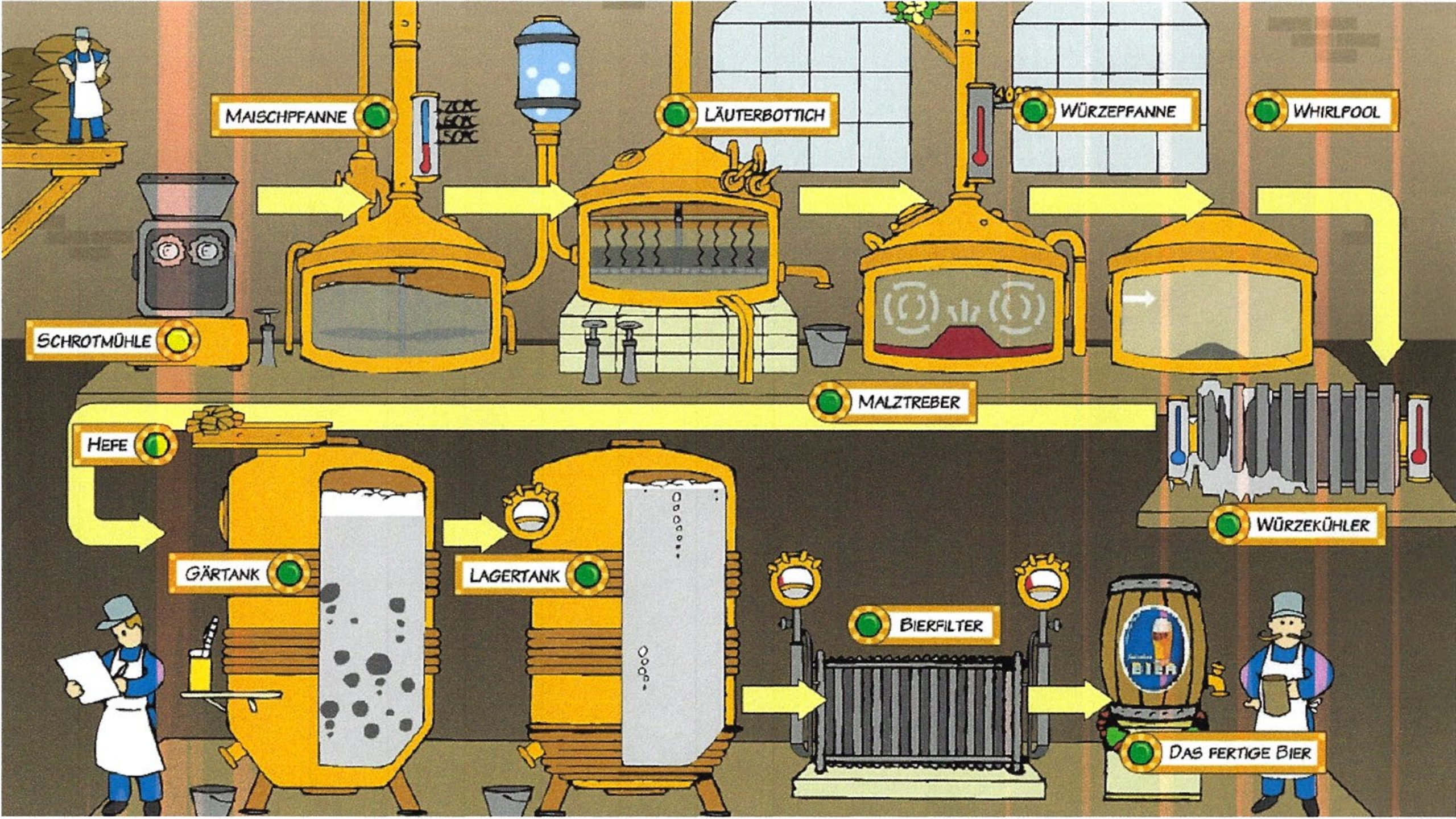
from yeast *Williopsis mrakii* AS-15

have been produced in brewery in:

- **Microtechnical scale and**
- **Technical scale**


BIOENGINEERING


aBrew jpBrew
Montag, 27. Februar 2006 09:54:46
Sudhaus
Schroterei
Maischepfanne
Lauterbottich
Würzepfanne
Whirlpool


MAISCHPFANNE
LÄUTERBOTTICH
WÜRZEPFANNE
WHIRLPOOL
SCHROTMÜHLE
MALZTREBER
HEFE
WÜRZEKÜHLER
GÄRTANK
LAGERTANK
BIERFILTER
DAS FERTIGE BIER

Evaluation of dermatological effects of cosmetic formulations

The evidence that reactive oxygen species are involved in the aging process and in the pathogenesis of many diseases as well as the indication that topical application and systemic administration of antioxidants has biological effects led to a great interest in the potential role of specific active substances in these effects.

Some studies showed that antioxidants, acting as photo protectives, could maintain or restore a healthy skin barrier.

Among the frequently used antioxidants in anti-aging products we can point out vitamin A, C and E derivatives as well as yeast antimicrobial proteins (YCPs).

Evaluation of dermatological effects of cosmetic formulations

On the other hand, a new tendency in cosmetic formulations is the use of biotechnological raw materials for antioxidant, immunomodulatory and photo protective purposes.

Moreover, yeast complex proteins extract (YCPs) with very low concentration of DL(+)lactic acid (in probiotic level) assuming a prominent role among biotechnological raw materials.

YCPs are rich in amino acids which have moisturizing properties, peptides, glycoproteins and polysaccharides (β-glucan). They can wound healing and cell renewal antimicrobial, antiallergy and probiotic effects.

Evaluation of dermatological effects of cosmetic formulations

Consequently, according to these previous studies, it can be suggested that

yeast complex proteins extract with supplements offers great potential for the prevention of

photo-aging, antimicrobial and oxidative stress diseases,

because despite its **immunomodulatory activity**, it presents antioxidant and antimicrobial

activity, can improve photo aged and healthy skin.

Now, there is a great tendency in the cosmetic area in the development

of **multifunctional** cosmetics. Therefore, it is very important to evaluate the performance of

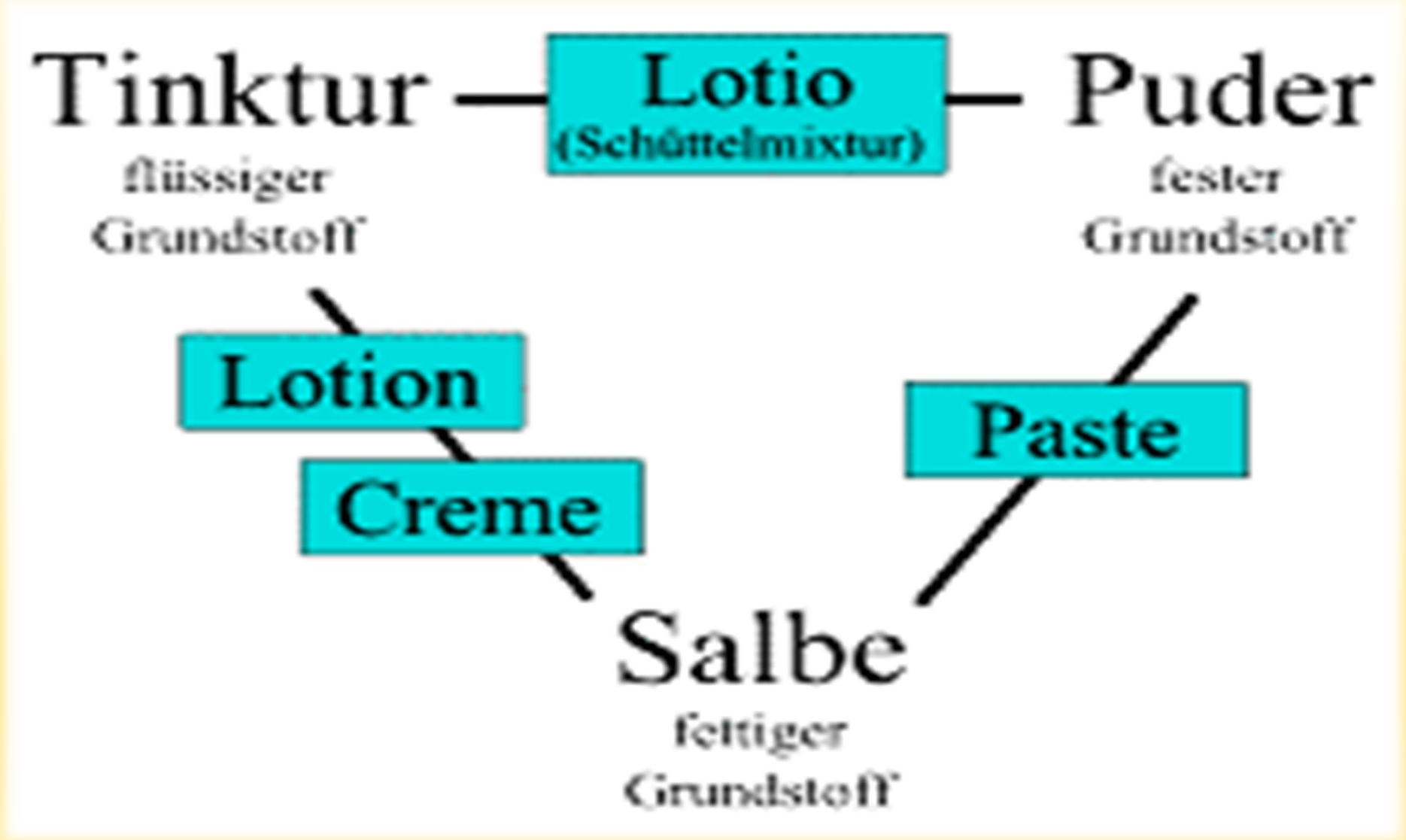
cosmetic products containing **YCPs extract with some special supplemnts**.

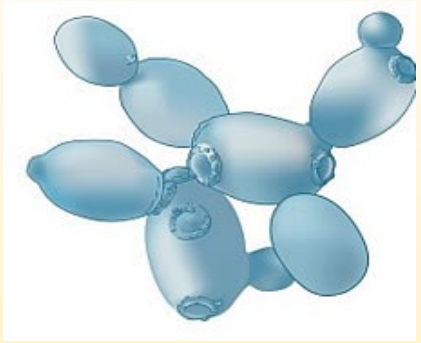
Evaluation of dermatological effects of cosmetic formulations

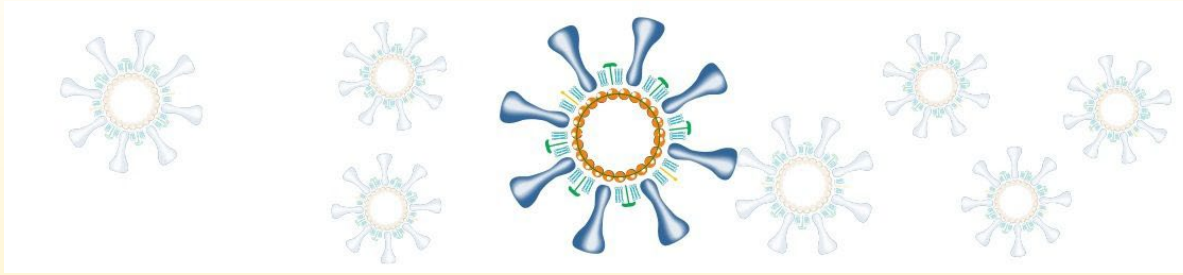
Based on these considerations, we evaluated the dermatological effects of cosmetic formulations containing **yeast complex proteins extract** and/or some specific supplements in order to improve skin conditions and to promote healthy skin.

Finally, complete evaluations such as the ones performed in our study are very important in the development of new cosmetic products to evaluate the best correlation between risk and benefit and to obtain **safe and antimicrobial cosmetic products**.

Dermatological effects in cosmetic formulations



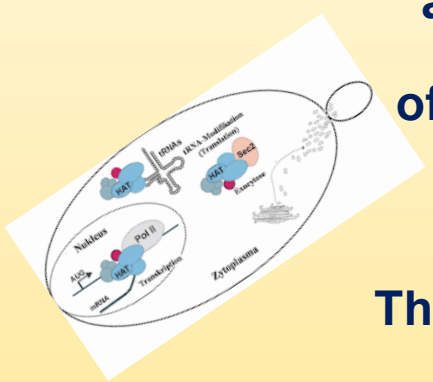




Yeast System as Probiotic Mikrobiom

Against Porcine & Respiratory Syndrom

Yeast System (in killer substrains *Kluyveromyces lactis*)



Kluyveromyces lactis **is a yeast which has the ability to assimilate lactose and convert it into lactic acid.** ***Kluyveromyces lactis*** **as a representative of the so-called killer yeasts, uses this strategy to gain a selective growth advantage in the competition for nutrients via microbial competitors.**

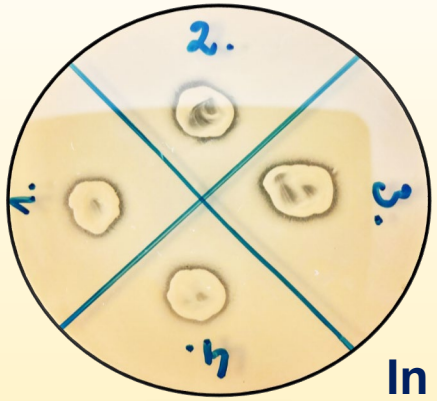
These can be blocked by the effect of zymocin in cell growth and cell cycle.

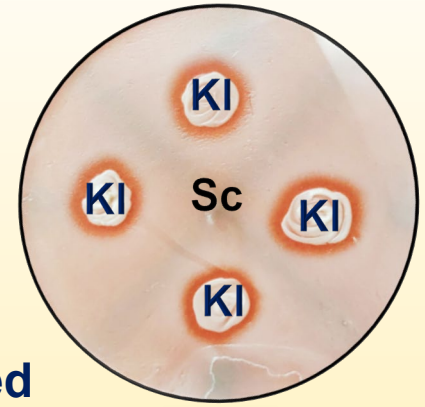
The ***Kluyveromyces lactis*** **toxin is plasmid-encoded and inhibits the multiplication of the baker's yeast** ***Saccharomyces cerevisiae*** **through an irreversible block in the G1 phase of the cell cycle.**

Sensitive target cells arrest without sprouting with a pre-replicative (1n) DNA content.

Despite the heterotrimeric (αβγ) structure of the native zymocin, its toxicity is primarily caused by the γ subunit, the so-called γ-toxin.

Yeast System (+ killer substrains)



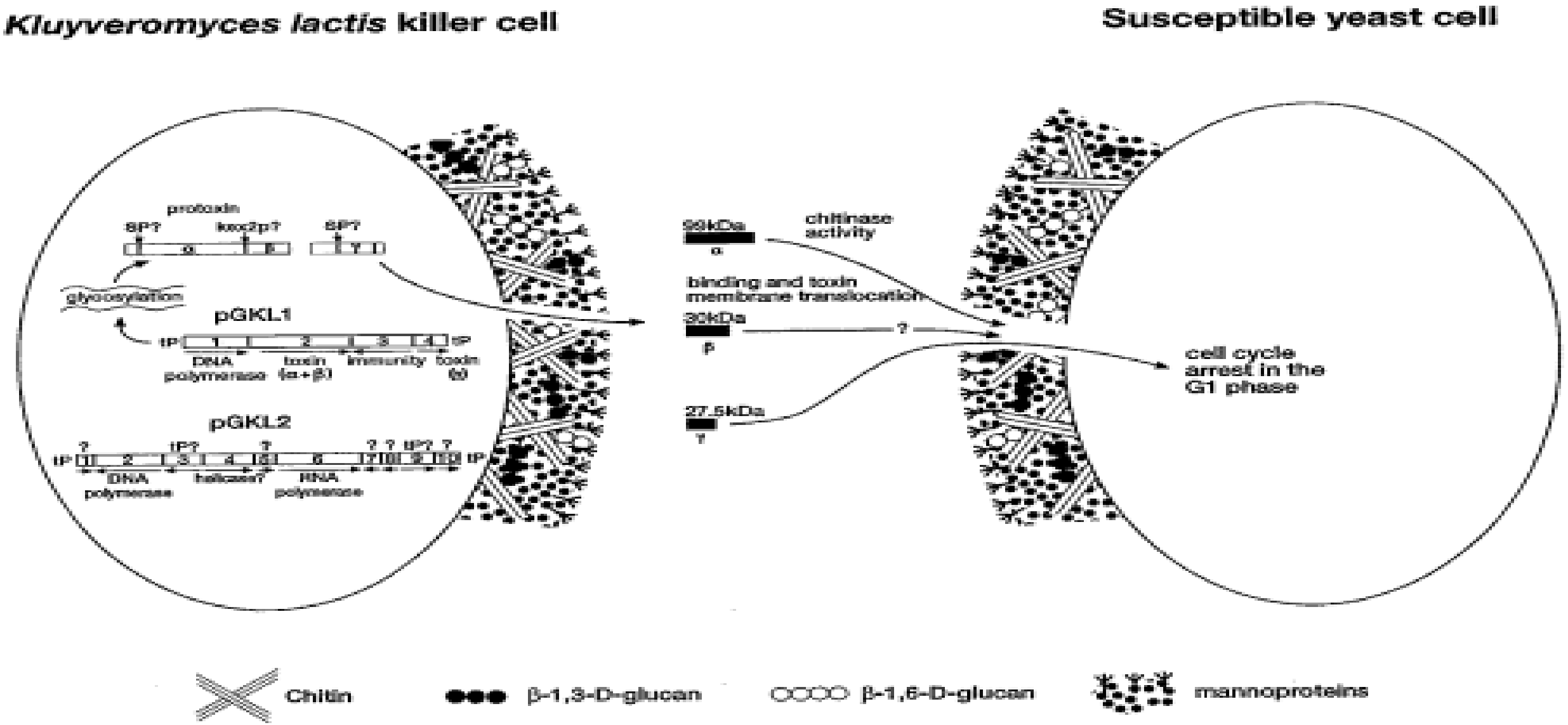


Secretion of cytotoxic substances by microorganisms is one strategy to combat microbial competitors.

In yeast, numerous such killer toxin systems, either chromosomally encoded or associated with dsRNA viruses or cytoplasmic dsDNA plasmids, have evolved. Biochemically, these toxins vary by receptor specificity, by being secreted as monomers or multi-subunit complexes and by their cytotoxic effects towards target cell proliferation. The lethal interaction between *Kluyveromyces lactis* and *Saccharomyces cerevisiae* constitutes a model pathosystem that relies on zymocin, a heterotrimeric protein toxin complex secreted by the dairy yeast *Kluyveromyces lactis,* which causes an irreversible growth arrest of sensitive yeast cells.

This killer phenomenon is encoded by a cytoplasmic pair of killer plasmids, pGKL1 (k1) and pGKL2 (k2), and ultimately causes a G1 (growing phase) block.

Kluyveromyces lactis with linear DNA plasmids pGKI1 & pGKI2



Yeast Killer System

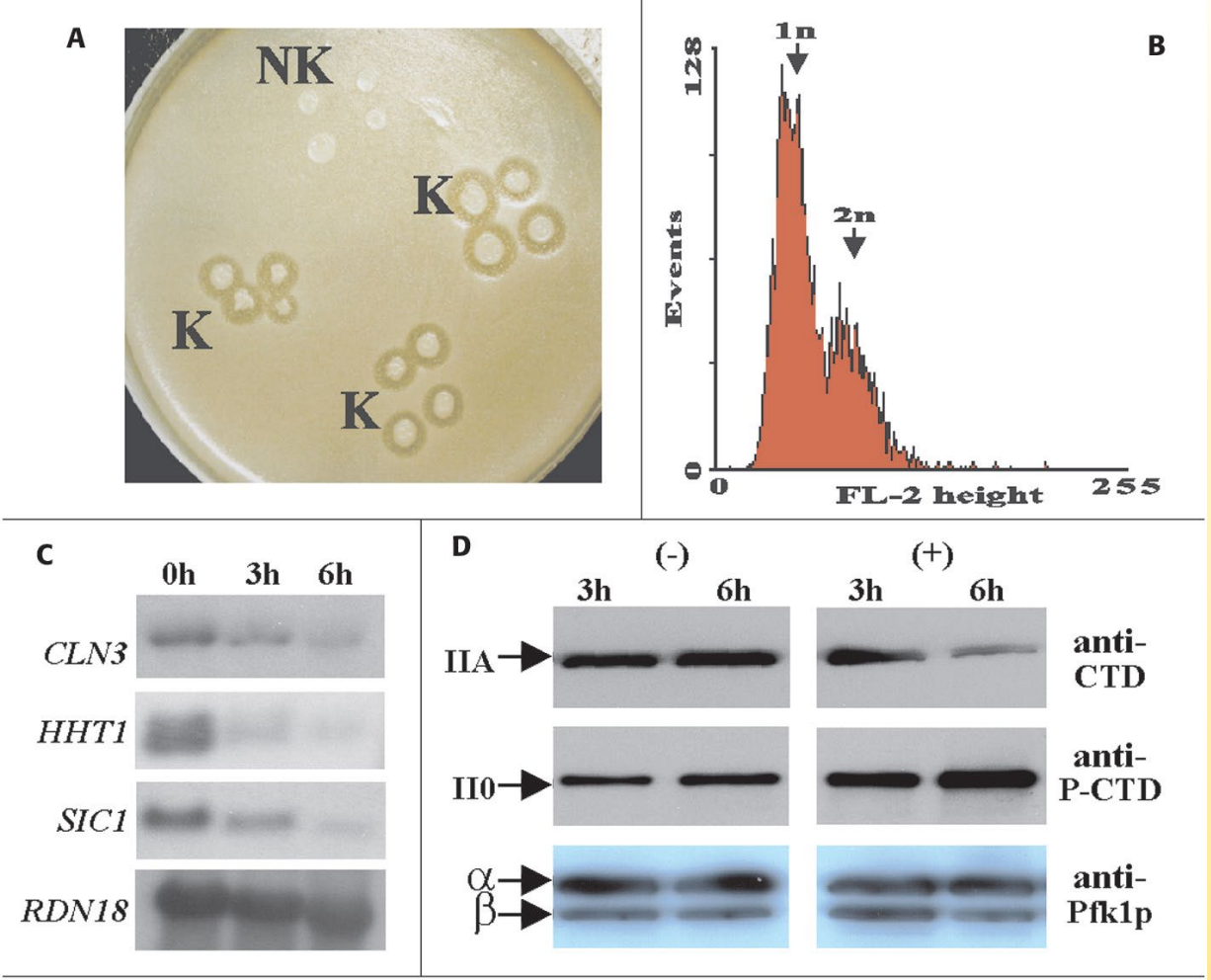


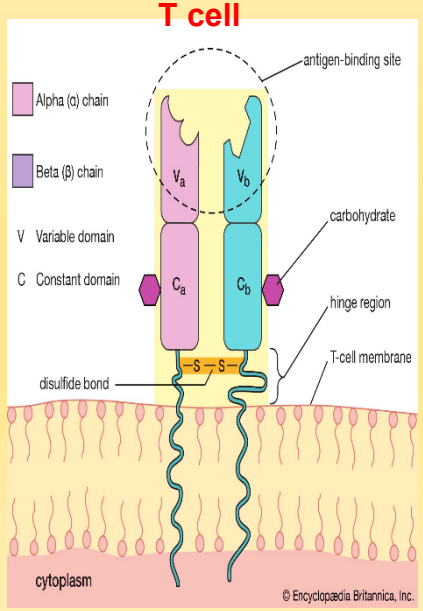
Abb. 1: Das Killersystem von Kluyveromyces lactis. (A) Killer- (K) und Nicht-Killerstämme (NK) wurden gegen S. cerevisiae inkubiert. Hemmhöfe machen die Wachstumsinhibition sichtbar. (B) FACS-Analyse. Zymocinbehandelte S. cerevisiae-Zellen akkumulieren in der G1-Phase des Zellzyklus mit 1n DNA-Gehalt. (C) Northern-Analysen an Zymocinunbehandelten (0h) und Zymocin-arretierten Zellen (3h bzw. 6h nach Zymocingabe). Identische Gesamt-RNA wurde auf Pol I (RDN18) und Pol II (CLN3, HHT1 und SIC1) Transkription hin untersucht. (D) Phosphorylierung der C-terminalen Domäne (CTD) der Pol II-Untereinheit Rbp1p. Proteinextrakte Zymocin-unbehandelter (–) und -arretierter Zellen (+) wurden mit Antikörpern, die hypo- (anti-CTD: IIA) von hyperphosphorylierter CTD (anti-P-CTD: II0) unterscheiden, im Westernblot analysiert. Als Ladekontrolle diente anti-Pfk1p zur Detektion der α- und β-Untereinheiten der Phosphofructokinase. (E) Arbeitsmodell zum Zymocin-Signalweg, der in Bindung an die sensitive Zielzelle (1), γ-Toxinimport (2) und intrazelluläre Kommunikation des γ-Toxins mit TOT (3) einteilbar ist. Zu den Genprodukten (Chs3-4p, Chs7p, Grx3p, Kti11-14p & Sit4p) siehe Text.

Kluyveromyces lactis application

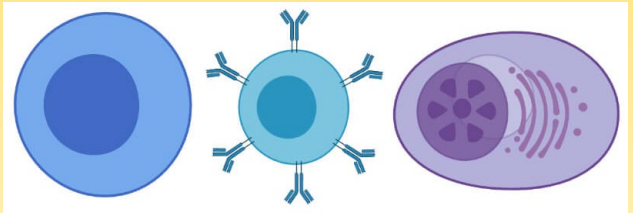
The mucosa of respiratory and reproductive tracts is the major route of respiratory infection. It is believed that generating mucosal immunity using vaccines is the best way to prevent that infection.

It has been reported that some recombinant yeast *Kluyveromyces lactis* can be administered orally and in this way it deliver proteins as antigen induce mucosal immune responses.

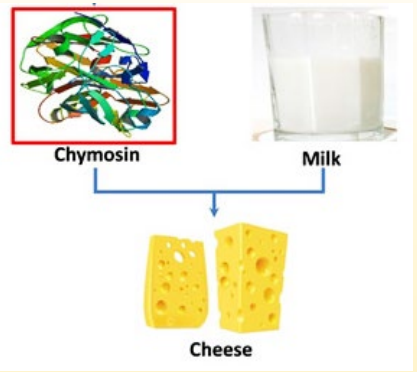
T cell



In the our study, we isolated natural *Kluyveromyces lactis* expressing antigen to respiratory syndrome and have evaluated its ability to induce (in our organism) B cell- and T cell – mediated immune responses after oral delivery.



B cell, lymphocyte



Kluyveromyces lactis application

The yeast system has been shown to have advantages over conventional systems as a vaccine vehicle.

For example, *Kluyveromyces lactis* is generally regarded as safe for animals and human beings. *Kluyveromyces lactis*, one of the most important non-*Saccharomyces* yeasts, has similar advantages to yeast *Williopsis mrakii*.

Additionally, *Kluyveromyces lactis* has a well-established track record of safe use in various food industry applications (chymosin in cheese production) and can efficiently express heterologous proteins. Moreover, components of its cell-wall such as β-1,3-glucan and mannan may have adjuvant activities. Thus, *Kuyveromyces lactis* might be a safe and ideal vaccine vehicle.

Kluyveromyces lactis application

Kluyveromyces lactis **has been used as a source of lactase (β-galactosidase), an enzyme that degrades milk sugar (lactose) and is necessary for production of lactose-free dairy products.**

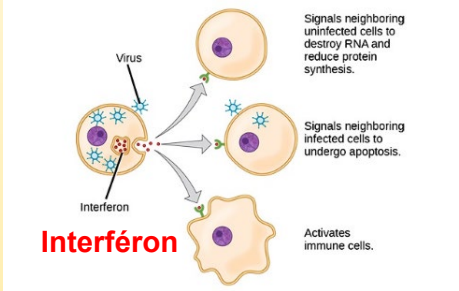
Kluyveromyces lactis **is best known for its use in commercial production of the milk-coagulating enzyme bovine chymosin.**

Today, over 40 proteins have been produced with *Kluyveromyces lactis*,illustrating its utility as an alternative yeast expression system. These proteins originate from bacteria, fungi, viruses, plants, and mammals, emphasizing the ability of *Kluyveromyces lactis* To efficiently produce a diverse range of heterologous proteins.

Kluyveromyces lactis application

The success of *Kluveromyces lactis* as a host for protein expression in the food industry suggests that this yeast could also be used for large-scale therapeutic protein production in the pharmaceutical industry.

In fact, numerous proteins of pharmaceutical relevance have been produced by *Kluveromyces lactis*.



The mammalian proteins interleukin 1-β, interferon αA, α-lactoglobulin, lysozyme, macrophage colony stimulating factor, serum albumin and insulin precursor have been secreted from *Kluyveromyces lactis* to high titer.

***Kluyveromyces lactis* is also proficient in the production of single-chain Fv antibodies and interesting native enzymes like inulinase, phospholipase B and chitinase.**

Kluyveromyces lactis application

In this context, *Kluyveromyces lactis* represents an interesting species.

While it is an attractive model for biotechnological procedures such as production of pharmaceuticals, yield of heterologous proteins.

This aerobic yeast is especially well-known for its ability to assimilate lactose.

Kluyveromyces lactis represents one of the leading yeast contributors of dairy products.

In *Kluyveromyces lactis*, the lactose assimilation process (in adaptive mode) relies on a well-known pathway comprising the *LAC4* and *LAC12* genes, which encode respectively a β-galactosidase and a lactose permease, as well as the galactose-lactose regulatory genes (*LAC9* and *GAL80*) and the galactose genes (*GAL1, GAL7* and *GAL10*).

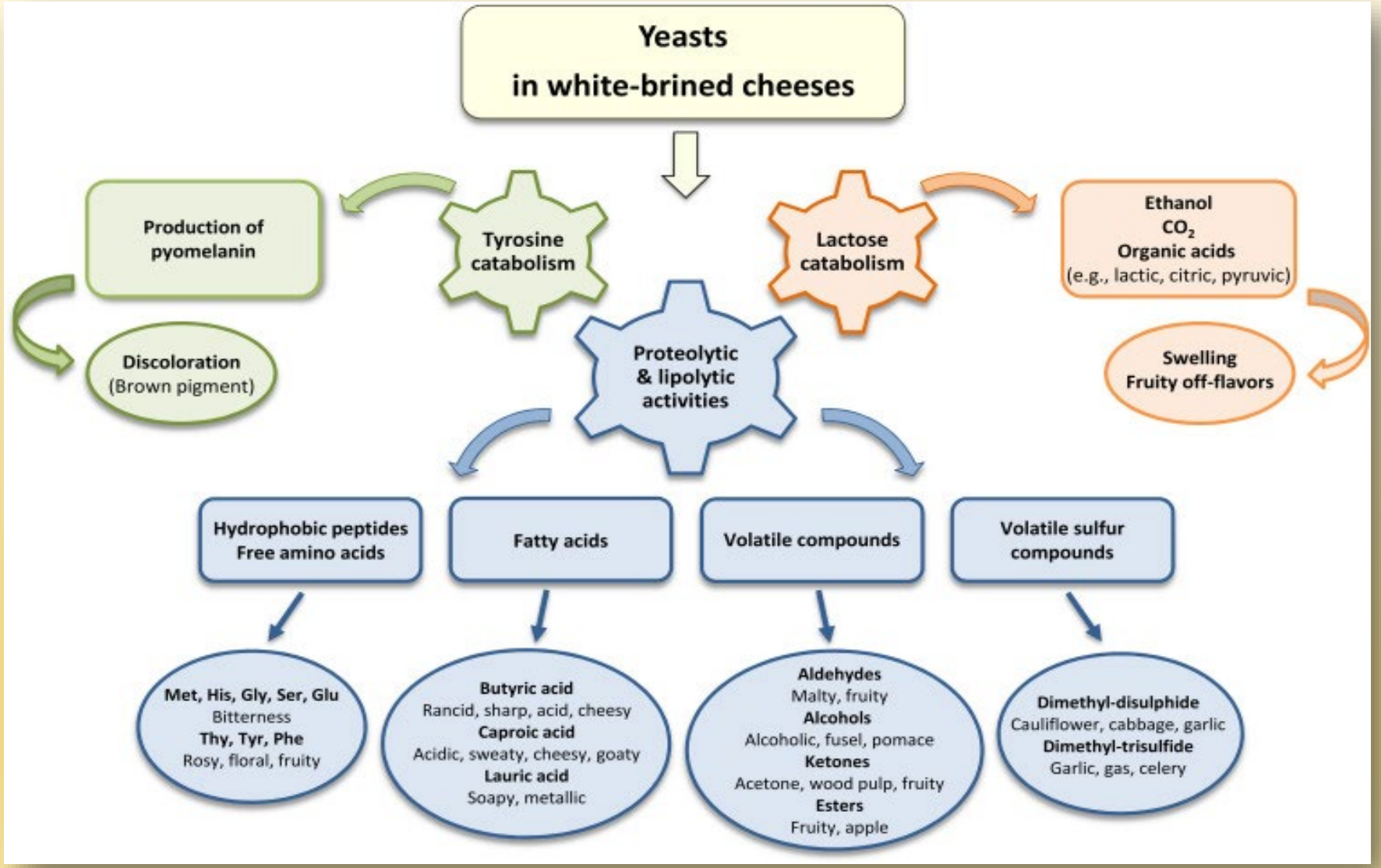
Kluyveromyces lactis application

Lactose-hydrolyzed milks also improve lactose intolerance symptoms in both children and adults. A by-product of lactose-hydrolyzed milk is increased sweetness due to the presence of free glucose.

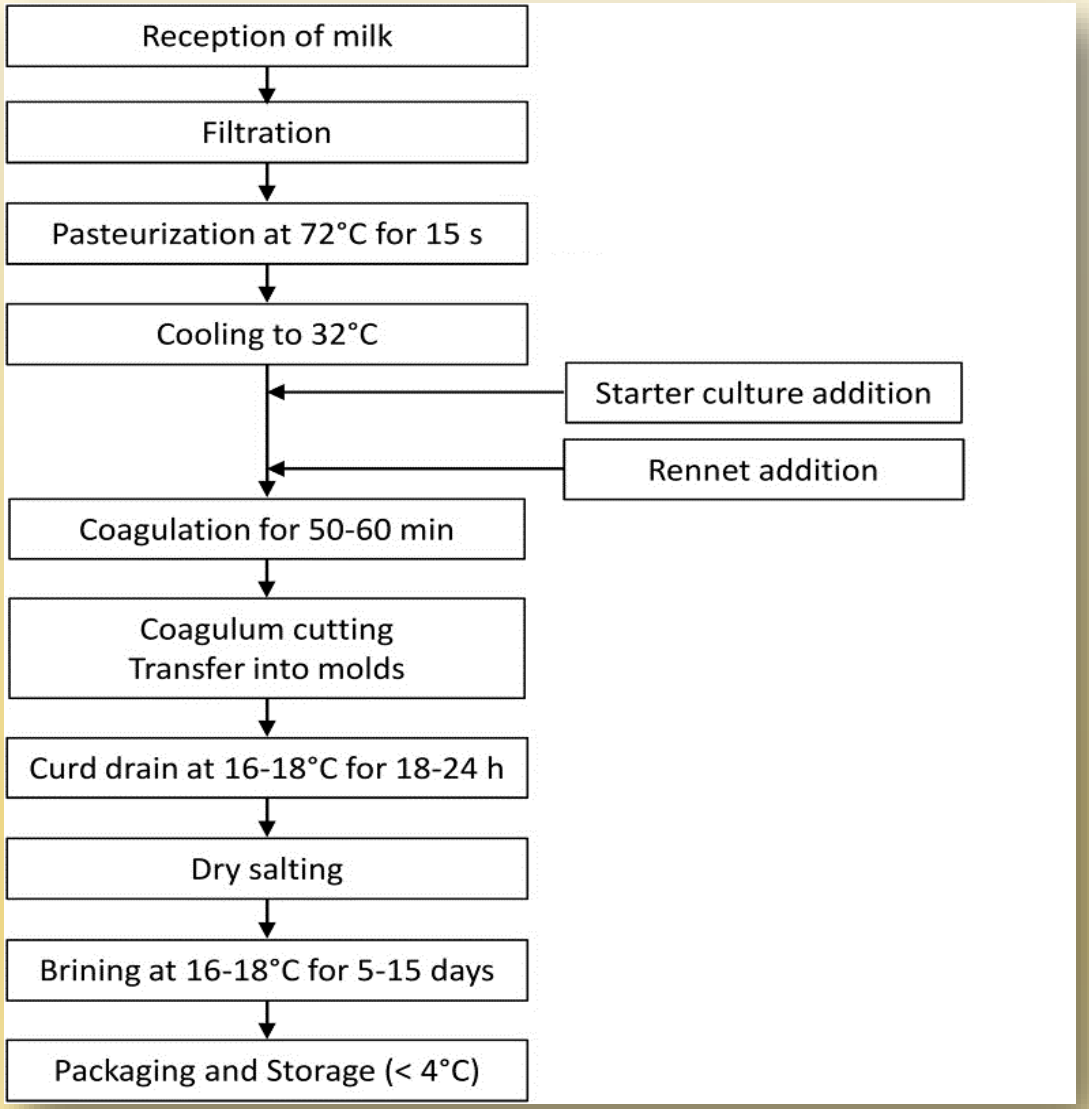
This increased sweetness may increase its acceptability in children.

The ability of *Kluyveromyces lactis* to metabolize milk constituents (lactose, proteins, and fat) makes them very important in cheese ripening and fermented milk products such as kefir, as they contribute to maturation and aroma formation. *Kluyveromyces lactis* is very active for the catabolism of peptides and amino acids (proteolytic), as well as fat (lipolysis), which leads to the production of a diversity of flavours.

Kluyveromyces lactis application



Kluyveromyces lactis application



Conclusion

- "Kluy-lac" drink (milk with *Kluyveromyces lactis*) is: 'A protein drinks a day'.
- Protein consists of amino acids (small protein particles), known as the "building blocks of life and health".
- "Kluy-lac" drink contains bioactive peptides, amino acids and metabolites with antimicrobial properties(such as α-glycopeptides, zymocin, L(+)-lactic acid and DL-racemic lactic acid). The yeast used in "Kluy-lac" drink hydrolyze lactose to L(+) and DL(+) racemic lactic acid,thanks to the enzyme β-galactosidase, therefore this drink is lactose-free with many positive (incl. antimicrobial) effects.
- The strengthening of the immune system is due to the specific α-glycoproteins produced by metabolism of the yeast used in "Kluy-lac" Drink.
- Another valuable active ingredient in "Kluy-lac Drink is α-lactoalbumin (α-LA), which is evaluable source of important amino acids and stimulates the body's immune response.
- The refreshing effect after fermentation in "Kluy-lac" Drink is due to the resulting carbon dioxide and ethanol.

Conclusion

- Therapeutic uses of “Kluy-lac” Drink are mainly due to its content of lactoferrin, immunoglobulins, growth factors such as branched-chain amino acids (i.e. leucine, isoleucine and vanillin) and micronutrients (potassium, calcium, iron, phosphate and B vitamins).
- The “Kluy-lac” drink formula used improves the health of skin and hair thanks to the amino acids and presence of B-complex vitamins (especially B1 and B6).
- “Kluy-lac” can be used both as a natural food or as a dietary therapeutically supplement for patients with the following problems:
  - Chronic bacterial, viral or fungal infections;
  - Weakening or deregulation of the immune system;
  - Digestive system problems (diarrhea, constipation, dyspepsia, flatulence, etc.);
  - Loss of muscle mass or excess weight of fat mass;
  - Calcium absorption disorders, calcium deficiency, e.g. by osteoporosis.

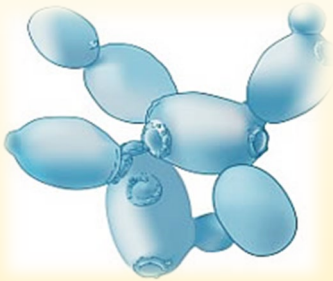
Conclusion

After homogenisation:

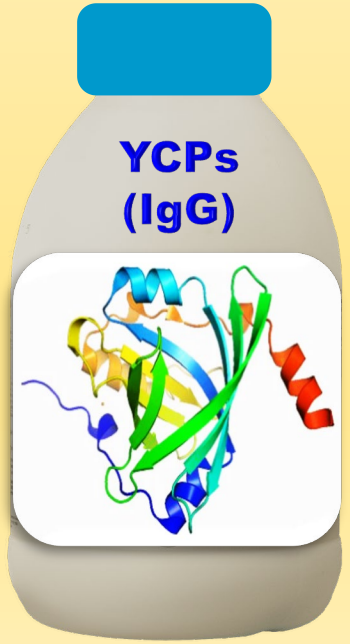

Zum Wohl!
Cheers!
Probiotic Drinks
Kluy-lac
Lactose free
Soft cheese
Lactose free

Immunity Test

IgG = YCPs = antigen



Thank you for your attention!



e-mail: anna.salek@t-online.de